BIOFOR' 02

Sustainable Forestry, Wood Products & Biotechnology

Vitoria-Gasteiz, Espagne
11-14 novembre 2002

COMPTE-RENDU DE MISSION

Philippe Vigneron
Jean-Marc Gion

CIRAD

Forêts
Programme
Arbres et Plantations

BIOFOR'02 – Sustainable Forestry, Wood Products & Biotechnology
Vitoria-Gasteiz, Espagne
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Compte-rendu de mission
Philippe Vigneron
Jean Marc Gion

Organisateurs : NEIKER - Instituto Vasco de Investigación y Desarrollo Agrario
Gouvernement Autonome Basque
Région Aquitaine

Jean Marc Gion et Philippe Vigneron ont participé à ce congrès et ont présenté une communication orale et un poster. Le CIRAD est par ailleurs co-auteur de la communication orale de Jacqueline Grima-Pettenati.

Vigneron Ph, Gion J-M & Verhaegen D, CIRAD Forêt. Montpellier (FRANCE) UR2PI-Congo : Genomic researches : new tools for genetic improvement of eucalypts in Congo. **Voir annexe et diaporama fichier vig et gion03.pdf**

J.M. Gion, M. Lalanne, C. Plomion , INRA : Wood proteomics in maritime pine. **Voir annexe et poster Pgion.pdf**

Jacqueline Grima-Pettenati, CNRS : Functional genomics of wood formation in Eucalyptus: a Southern Europe project. **Voir fichiers GP1.jpg et GP2.jpg**

Les résumés (1 à 3 pages) des communications orales et des posters sont disponibles auprès de Ph. Vigneron ou J.M. Gion.

Les objectifs du congrès sont exposés ci-dessous (from the Symposium announcement)

The congress aims to address, in the broadest sense, possible applications of biotechnology for sustainable forestry and for improving quality of wood products.
The importance of Biotechnology for forestry and forest products is growing rapidly. The recent scientific innovations in this field offer increasing possibilities of applications. These facts have motivated the Organizing Committee to organize this congress to share and disseminate novel achievements obtained in R&D projects and to identify gaps for future research.
Furthermore this congress represents a platform where scientists and industrial partners can meet, discuss industrial needs, problems and possible solutions and can establish collaborations. For this purpose, the congress will include an active participation of enterprises from the forest sector, of wood processing industries and biotech companies.
Moreover, within the frame of this congress, facilities will be given to hold independent project meetings or workshops for interested groups. This option is particularly atractive for establsihing collaborations among participants in view of the approaching ***Framework VI Programme of the EU****.*
Several members of the Management Committee ***COST action E28*** *"Genosilva: European Forest Genomics Network" of the European Commission participate in this event. Furthermore, the* ***National Forest Genomics Network*** *of Spain is under constitution and the*

partners will make relevant contributions. In order to underpin the importance of Genomics in Forest Research, extended results of the ***QoL R&D project*** *"UHD map of Coniferous species (QLK5-1999-01159)" will be presented involving several conifer species.*
Within the congress ***Satellite Meetings*** *will be held: - The Genetic Resources Group and the management committee of* ***IEFC*** *(European Institute of Cultivated Forests) will meet as part of the IMACFORD project - task B: Development of an integrated research network for the sustainable management and utilisation of fast growing forest resources in Europe.Furthermore, the working group:* ***Management of Forest Habitats*** *which belongs to* ***Natura2000*** *will present and discuss results and experiences.*

Trois types de réunions ont été tenues de façon concomitante . Les industriels et les producteurs basques et aquitains ont débattu des aspects transformations – commercialisation d'une part et des aspects certification d'autre part. Les chercheurs ont présenté et discuté leurs travaux de laboratoire. Il faut regretter que malgré les buts poursuivis, il n'y ait pas eu suffisamment de rencontres entre les différents acteurs : chercheurs, producteurs, transformateurs. Ce compte-rendu de mission rapporte l'état des recherches sur Eucalyptus tel qu'il a été présenté par les différents intervenants.

Les travaux sur Eucalyptus

Multiplication végétative

Les papiers présentés montrent l'importance croissante de l'embryogénèse somatique et des graines artificielles pour le déploiement industriel des conifères, principalement aux US, Canada et Nouvelle Zélande. Le système est au point et utilisé en routine. Rien n'a été présenté sur Eucalyptus ou autres feuillus.

L'Afocel a présenté un poster sur la cryoconservation d'espèces diverses, dont les eucalyptus : E. Dumas et al. « Cryopreservation: a powerful tool for the management of genetic resources for breeding and preservation purpose ».

Transformation génétique

De nombreux travaux prometteurs ont été publiés sur conifères, bouleau et peuplier mais l'Eucalyptus reste le parent pauvre.
Les travaux sur la transformation génétique des eucalyptus sont relativement anciens mais se sont toujours heurtés à la phase clef qui est la régénération d'une plante entière après l'obtention d'un cal cellulaire transformé. Visiblement, si on observe la transformation de cals cellulaires, l'écueil de la régénération est toujours présent.

- Le papier présenté par Gallego (Université de Vigo, Espagne) sur la transformation de E. globulus n'est pas concluant : il présente une étude des différentes conditions de transformation par infiltration sous vide avec Agrobacterium et obtient des cultures de feuilles ou de bourgeons exprimant le gène GUS (j'allais dire une fois de plus) mais reste très évasif sur la régéneration.
- Le poster « Efficient transformation systems for testing wood quality genes in hardwoods » de la compagnie privée ArborGen (South Carolina, USA) par MA Hinchee, Briggs T, Chang S, Frampton KA, Gause KC, Richardson P, Thomas RD, Wilde HD est un peu plus optimiste. ArborGen semble avoir fait des progrès significatifs dans le

domaine de la régénération de clones d'E. grandis, d'hybrides E.grandis x urophylla et d'E. camaldulensis après transformation avec des constructions génétiques destinées à modifier et amémliorer la qualité du bois pour l'industrie papetière. Ils ne donnent pas de détail sur l'identité des génes réprimés ou surexprimés. Le nombre de clones obtenus est encore faible (2 grandis x uro) et seuls 10 % des clones se régénèrent. Les premiers essais de terrain sont en cours aux US avec un total de 75 clones, essentiellement E. camaldulensis.

GENOMIQUE et BIODIVERSITE

Les travaux présentés lors de cette session et de la suivante (Industrial needs and applications) tournent autour de trois axes complémentaires de recherche : la cartographie génétique, la recherche de QTL et l'analyse du transcriptome.
Les travaux de cartographie sur les arbres forestiers utilisent de nombreux types de marqueurs (SSR, EST, gènes candidats, RAPD, AFLP...) . De nombreuses cartes génétiques saturées sont maintenant publiées tant pour des conifères que des feuillus. Ces cartes génétiques servent au positionnement des QTL puis des gènes candidats. Deux problèmes majeurs se posent :

- la taille réduite des populations de cartographie (familles de pleins ou demi frères) qui ne permet qu'une estimation très grossière des taux de recombinaison entre marqueur et donc une localisation très imprécise des marqueurs sur le génome,
- la faiblesse statistique des dispositifs au champ et la difficulté qu'il y a de distinguer les composantes génétiques et environnementales.

Ceci conduit généralement à une grande imprécision de la localisation des QTL et de l'intensité de leurs effets, au faible nombre de QTL détectés eu égard au caractère polygénique des propriétés mesurées, à la détection de faux QTL...
Pour différentes raisons, dont la rapidité de croissance, l'importance des dispositifs de terrains, la faible taille du génome, l'existence de familles de pleins frères présentant des effectifs importants, la grande diversité allélique... les travaux sur Eucalyptus sont maintenant parmi les plus avancés en la matière.

Les quatre partenaires du projet « Génomique fonctionnelle de la formation du bois d'Eucalyptus » ont fait le point sur l'état de la génomique Eucalyptus en Europe. Ce projet associe ENCE (Espagne), RAIZ (Portugal), CNRS (Toulouse, France) et le CIRAD. Il est présenté par Jacqueline Grima-Pettenati.

- JACQUELINE GRIMA-PETTENATI
Functional genomics of wood formation in Eucalyptus: a Southern Europe project.

L'objectif du projet est d'identifier (CNRS) et de cartographier (ENCE, RAIZ et CIRAD) des gènes candidats (GC) et des QTL responsables des variations des propriétés du bois dans l'objectif de développer de la sélection assistée par marqueurs. Des GC « expressionels » (c'est à dire exprimés) ont été isolés en utilisant la technique d'hybridation soustractive (Suppression Soustractive Hybridization) entre feuilles et xylème. Une collection de 2700 EST (pour Expressed Sequence Tag) a été obtenue. La séquence des EST permet dans un certain nombre de cas de trouver, par comparaison à des banques de données, le gène en cause. Ce séquençage est en cours et certains gènes sont maintenant disponibles pour la cartographie (qui se fera à Montpellier sur la carte de la famille 14.144 x 9.21).
Afin de cibler au mieux les gènes spécifiquement exprimés dans le xylème, l'étude se poursuit en comparant divers types de xylèmes : bois juvénile/bois adulte, bois tendu/bois normal...

Tous ce travail fait l'objet de la thèse d'Etienne Paux.

- CRISTINA MARQUES
Forest Biotechnology "To Go" at RAIZ

RAIZ est un institut de recherche privé dont la fonction est d'aider à la compétitivité de la filière pâte et papier du Portugal. L'un des thèmes de recherche est la sélection de clones d'Eucalyptus globulus. RAIZ utilise les marqueurs pour le suivi de la diversité génétique de la population de clones et l'identification des variétés. Dans le cadre de notre partenariat, leur premier travail est de faire le tri dans les séquences exprimées dans le xylème. Il est en effet difficile et probablement inutile de cartographier les 2700 séquences. De nombreux gènes exprimés n'ont probablement rien à voir avec les propriétés du bois. En se basant sur la littérature existante, les bases de données génomiques, RAIZ a pour l'instant sélectionné 163 gènes (dont 80 prioritaires) sur la base de leurs fonctions physiologiques ou métaboliques supposées dans la formation du bois. Ce sont ces gènes qui seront cartographiés sur E. globulus et E. urophylla x grandis et dont on cherchera les colocalisations avec des QTL.

ROBERTO ASTORGA & GABRIEL TOVAL
Industrial Applications of Biotechnology at ENCE

La division forestière d'ENCE gère 150 000 ha, essentiellement des Eucalyptus, en Espagne et en Uruguay. Ils produisent environ 3 millions de m3 de bois par an. La division « Pâte » produit environ 1 million de tonnes de pâte Kraft par an. Le programme d'amélioration travaille essentiellement sur E. globulus. Les marqueurs moléculaires (SSR) ont été utilisés pour l'analyse de la diversité génétique des populations de base, de celle de la population d'amélioration ainsi que pour le contrôle de l'identité des variétés déployées. Ils sont en train de développer une nouvelle banque de microsatellites (SSR) et commencent leur cartographie. Des QTL seront recherchés pour la résistance à la sécheresse, l'enracinement et la qualité du bois (avec le groupe des 4 partenaires). Nous sommes en discussion avec Roberto Astorga pour l'utilisation de leurs microsatellites.

PHILIPPE VIGNERON
Genomic researches : new tools for genetic improvement of eucalyptus in Congo

Nous avons brossé un tableau des différents aspects de la recherche en génétique moléculaire sur Eucalyptus. Le résumé de la présentation orale est donné en annexe. La présentation diaporama est disponible.

La présentation orale a été axée sur :
- l'importance de disposer à la fois de nombreux marqueurs mais aussi, et c'est crucial, de familles de grande taille pour pouvoir construire des cartes génétiques à la fois saturées (génome bien couvert) et précises en terme de positionnement des marqueurs les uns par rapport aux autres ;
- la nécessité d'avoir une mesure fiable des caractères pour lesquels on recherche des QTL ;
- l'importance de disposer de généalogies complexes et diversifiées pour multiplier les chances de mise en évidence de QTL, de trouver du polymorphisme utile dans les populations d'amélioration, de tester la valeur des allèles dans des milieux différents ;
- l'importance des collaborations pour développer des travaux en génomique fonctionnelle (cf. notre collaboration ENCE-RAIZ-CNRS-CIRAD) ;

- l'utilité d'une carte consensus des principales espèces du sous genre Symphyomyrtus, travail auquel le CIRAD, ENCE et RAIZ vont s'atteler en utilisant des marqueurs communs (SSR et EST).

Les deux dernières diapos ont servi à montrer en quoi le schéma de SRR du Congo constituait un outil privilégié pour ces recherches et leurs applications.

Réunion ENCE - RAIZ – CIRAD - CNRS

En marge du congrès, les quatre partenaires se sont réunis pour discuter des travaux en cours. Etienne Paux doit soutenir sa thèse en 2004. Il aura d'ici là constitué les banques d'EST associées aux divers types de xylèmes : juvénile vs adulte et cinétique de la mise en place du xylème tendu vs normal. Le protocole d'analyse de cette cinétique a été simplifié et limité à 3 dates.

Le travail bibliographique réalisé par RAIZ permet de faire un tri a priori des EST à cartographier. La construction de primers sera faite très prochainement en coordonant les efforts de RAIZ et du CIRAD afin d'éviter les doublons. Leur cartographie sera réalisée sur E. urophylla x grandis au cours de l'année 2003.

Il a été par ailleurs décidé de saisir toute opportunité de financement pour poursuivre et étendre notre collaboration. Le projet EUCAGENE sera revu et augmenté (protéomique, banque BAC ?) puis soumis à financement dès que possible.

Programme et communications

Lundi 11 novembre

Session: MICROPROPAGATION and PHYSIOLOGY

ROBERTO RODRIGUEZ –University of Oviedo, Spain
Keynote on Micropropagation and Physiological Aspects

JENNY AITKEN
Somatic embryogenesis and organogenesis of conifers

JAN DICK
Genetic bases of adventitous root formation.

ARMAND SEGUIN
Molecular approaches for a better understanding of the defense response in forest tree species.

BORJA DIEGO
Age- and phase-change-related gene expression in Pinus radiata D. Don.

MARÍA BERDASCO
DNA methylation analysis useful for characterizing product quality: the example of Pinus radiata d. Don in vitro tissue culture.

CARMEN DIAZ-SALA
Molecular and cellular approaches to the study of maturation-related decline of adventitious root formation

SÓNIA GONÇALVES
Identification of genes differentially expressed during embryigenesis in maritime pine (Pinus pinaster).

CARLOS RAMIREZ
Regeneration of Mexican pines by somatic embryogenesis

CONCEPCIÓN ÁVILA
Molecular biology of ammonium metabolism in early stages of pine development

Session: GENETIC TRANSFORMATION in forest species

CHRISTIAN WALTER – Forest Research Inst., New Zealand
Keynote on Genetic Transformation in Forest species: Modern Biotechnology as a new and promising tool in plantation forestry: Current status and future challenges

TREVOR FENNING
Perspectives and Prospects for Forest Biotechnology in Europe

ZIV SHANI
Plant growth modulation by Arabidopsis thaliana endo-1, 4-B-Glucanase (cell) in transgenic plants

Mardi 12 novembre

Session: GENETIC TRANSFORMATION in forest species (suite)

MATTHIAS FLADUNG
Towards the safe use of transgenic trees

TUIJA ARONEN
Transgenic silver birch (Betula pendula) with lignin modifications

ODED SHOSEYOV
Recombinant cellulose crosslinking proteins: novel fiber-modification biomaterials.

PEDRO PABLO GALLEGO
Genetic transformation of Eucalyptus globulus

MATTHIAS FLADUNG
Isolation of tree-specific genes and promoters by a transposon-tagging approach

KRYSTYNA KLIMASZEWSKA
Transgene integration patterns and expression levels in transgenic lines of three spruce species

SUBHASH MINOCHA
Genetic manipulation of polyamine metabolism in poplar

JEAN-FRANCOIS TRONTIN
Current prospects and limits to genetic transformation of Pinus pinaster Ait. using somatic embryogenesis as regeneration way

SUSANA TERESO
Establishment of a genetic transformation system in maritime pine (Pinus pinaster Sol.)

CHRISTIAN WALTER
Wood cells in the Petri dish: cell differentiation, secondary cell-wall formation and genetic transformation of secondary cell wall forming tissue of Pinus radiata d. Don.

Session GENOMICS and BIODIVERSITY

LADISLAW PAULE -Faculty of Forestry, Technical University, Zvolen, Slovakia
Keynote: Genetic diversity and differentiation of principal tree species

ENRIQUE RITTER - NEIKER, Spain
Keynote: Advances and challanges in Forest Genomics

ENRIQUE RITTER
Construction and application of a multifunctional and saturated genetic map for coniferous species

TORSTEN MARKUSSEN
QTL analysis for wood quality and growth traits in the P. pinaster reference population

ANA ARAGONES
Construction of a genetic map and QTL analyses in Pinus radiata

DANIEL PRAT
Linkage mapping and QTL analysis for growth of young Pseudotsuga menziesii plants

VIRGINIE ACHERE
Construction of linkage maps and application in Norway spruce (Picea abies)

TORSTEN MARKUSSEN
Construction of Linkage maps and QTL analyses in Pinus silvestris

DANIEL PRAT
Molecular dissection of growth and wood quality traits in larch

GUILLAUME BESNARD
Compared transmission of genomic blocks in Norway spruce [Picea abies (L.) Karst] progenies obtained under two different environmental conditions.

FRANCISCO J. RUIZ CANTON

Transcriptone analysis of wood formation in maritime pine

JEAN-MARC FRIGERIO
Seeking candidate genes for water deficit adaptation in maritime pine by transcript expression profiling

AINHOA LÓPEZ
Characterization of selected Pinus pinea L. Trees

Mercredi 13 novembre

Session INDUSTRIAL NEEDS and APPLICATIONS

LUC HARVENGT - AFOCEL, France
Keynote: Forest, Industry and Biotechnology. Needs, difficulties and some solutions for the future.

PRAMOD GUPTA - WEYERHAEUSER, USA
Keynote: Biotechnology of somatic embryogenesis, industrial application and implementation of this technology for reforstation

ENRIQUE TORIJA - BECKMANN
Strategies for Automating Nucleic Acid Preparation

CARLA PETRARROLI - ABI
High throughput marker analysis

GONZALO FIR PO - ABI
New Trends in BIOINFORMATICS

JORDI NAVARRO - AMERSHAM
Latest developments in high throughput 2D Proteomics

Les présentations des 4 partenaires du projet de développement de banque d'EST xylème d'eucalyptus

JACQUELINE GRIMA-PETTENATI
Functional genomics of wood formation in Eucalyptus: a Southern Europe project.

CRISTINA MARQUES
Forest Biotechnology "To Go" at RAIZ

ROBERTO ASTORGA & GABRIEL TOVAL
Industrial Applications of Biotechnology at ENCE

PHILIPPE VIGNERON
Genomic researches : new tools for genetic improvement of eucalyptus in Congo

WOLFGANG SCHUCH
Commercial introduction of elite conifer germplasm in forest plantations using somatic embryogenesis.

YILL-SUNG PARK
Implementation of industrial clonal forestry using conifer somatic embryogenesis in eastern Canada

ALAIN RIVAL
Large scale micropropagation of tropical tree crops: oil palm as a case study

BEATRIZ CUENCA
Cork oak phenotypic selection based on cork production and quality, biotechnological propagation and comercial application.

WORKSHOP: SCIENCE MEETS INDUSTRY

POSTER PRESENTATIONS

H-L. Pasonen, Pappinen A, Degefu Y, Timonen S, Brumos J
Effects of a sugarbeet chitinase 4 gene on mycorrhiza formation in transgenic silver birches (Betula pendula Roth) in vitro

S-K Seppänen, Pappinen A, Vahala J, Teeri T, von Weissenberg K
Modification of lignin by antisense suppression of 4CL in forest trees.

Z. Lorenzo, Soto A, Gil L
Utilisation of nSSR for Quercus ilex x Quercus suber hybrids identification.

O. Nicolaou, Parkes BD
High-throughput gene testing in radiata pine.

BD Parkes, Sathyapala SK, Gough K, Urangia RM, Grunwell J, Dorey R
A new age in forestry genetics: radiata pine somatic embryogenesis clones deployed in commercial plantings.

PA Balk, Verkerk-Bakker B, Konings MCJM, van der Geest AHM & van Wordragen MF
Differential expression of dehydrin genes in relation to dormancy development and release in bud tissue of Pinus sylvestris.

P. Alonso Rodriguez et al.
Cytokinins and morphogenesis in P. pinea cotyledons.

S. Espinel et al.
The P. radiata breeding programme in the Basque Country

D. Breton et al.
Effect of cultural methods on proliferation and their after-effect on maturation of maritime pine (Pinus pinaster) somatic embryos

E. Dumas et al.
Cryopreservation: a powerful tool for the management of genetic resources for breeding and preservation purpose

R. Hasbún et al.
Ageing and architecture determine specific DNA methylation profiles in trees: Application to selected tree propagation.

C. Walter, T. Pearson
Expression of a new gene in transgenic Pinus radiata in a field trial

S. Deroles, Walter C, Lill T & Javellana J
Biolistic transformation of Pinus radiata using a particle inflow gun

J. Charity, L. Holland, L. Grace, C. Walter
Expression of BAR, NPTII and UIDA in transgenic Pinus radiata after Agrobacterium-mediated transformation.
J-F. Trontin et al.
Bacterial strain, embryonal-suspensor masses (ESM) genotype and age affect transformation efficiency of Pinus pinaster Ait. via Agrobacterium tumefaciens

S. Jeandroz et al.
Mitochondrial DNA variation in french populations of Norway spruce (Picea abies [L.] Karst).

S. Espinel et al.
Genetic origin of the oak reforestations in the Basque Country.

M Fladung, M Kaldorf, W Gieffers, B Ziegenhagen, S Kumar
Field analysis of transgenic aspen

S Kumar, M Fladung
Transgene integration in Populus.

H Hönicka, M Fladung
Transformation of Populus with genes inducing male or female sterility

F Deutsch, J Kumlehn, M Fladung
Induction of haploid poplar through microspore culture

A. Ramboer et al.
Identification and characterisation of differentially expressed genes involved in anoxia root of sessile and pedonculate oaks.

Ma.T.Martin et al.
A modified ACM medium to maintain and in vitro collection of Populus tremula.

I. Creanga et al.
Quercus biodiversity analyzed by means of leaf fractality

M. Goicoechea et al.
Role of eucalyptus Myb factors in vascular tissues formation.

S. Marucci, Lucca F, Hopp E. et al.
Use of Molecular markers in Eucalyptus dunnii and E. grandis breeding programmes from Argentina.

N. S. Nehra et al
Method for production and regeneration of transgenic loblolly pine clones from diverse elite families

Pablo Goikoetxea et al.
Gene flow in Oaks

A. Ramboer et al.
Development of microsatellite markers in maritime pine

A. Ramboer et al.
Molecular methods for oak wood forensics

J.M. Gion, M. Lalanne, C. Plomion
Wood proteomics in maritime pine

Celia Fernandez et al.
Somatic embryogenesis in mature Quercus robur trees

D. Lopez-Vela, Hemández I et al.
Induction of somatic embryogenesis in half-sib families of selected Quercus suber trees

K. Hoefig, Moyle R.L, Putterill J, Walter C.
Tools to genetically engineer male sterility in Pinus radiata / expression analysis of four male cone promoters in Arabidopsis.

L.D. Phillips, Moody J., Geddes B., MacAskill U., Narayan R., Wagner A.
Expression studies of Reporter and Selection transgenes in Biolistically transformed Pinus radiata

M. Valbuena, S. C. González –Martínez, Á.Soto, C. Collada, P. Goikoetxea, L. Gil
Genetic relatedness and parentage analysis in a Quercus petraea and Quercus pyrenaica mixed oak stand in central Spain

MA Hinchee, Briggs T, Chang S, Frampton KA, Gause KC, Richardson P, Thomas RD, Wilde HD
Efficient transformation systems for testing wood quality genes in hardwoods

HD Wilde, Foutz KR, Thomas RD, Zhao Y, Hinchee MA
Transgenic eastern cottonwood (populus deltoides) with resistance to als-targeting herbicides

Kellison R, McCord S, Hinchee M
A guiding organization for forest biotechnology.

C. Ramírez-S., Soltero-Q. R, Santacruz-R. ME, Baena-A. R, Ávila-S. JE, González-Á. V, Pelayo-O. C.
Production of embryogenic tissue in mature zygotic embryos of Pinus maximartinezii rzedowski.

Rafael A. Cañas, Francisco M Cánovas, Francisco R. Cantón.
Accumulation pattern of asparagine synthetase during the initial stages of pine development.

Herrán A, Estioko L, Rodriguez MJB, Becker D, Lebrun P, Billote N, Baudouin L, Kullaya A, Bourdeix R, Konan JL, Barker JHA, Aldam C, Rohde W, Ritter E.
Exploitation of high density DNA marker maps in coconut.

Volosyanchuk, RT, Smulders, M.J.M.
Genetic diversity in Ukrainian natural populations of black poplar.

Volosyanchuk R, Los S, Yatsyk R, Hayda Yu, Polupan A., Bohomolov V, Kuznyetsova T, Neyko I, Gout R, Shvadchak I.
Inventory of genetic resources of broadleaved forest tree species in Ukraine

Billote N, Marseillac N, Risterucci A-M, Asmady, Herrán A, Singh R, Amblard P, Durand-Gasselin T, Brottier P, Courtois B, Cheah SC, Rohde W, Ritter E
Reference and SSR multi-parent linkage maps for molecular breeding in oil palm (Elaeis guineensis Jacq.).

Kellison R, McCord S, Hinchee M
A guiding organization for forest Biotechnology.

P Garnier-Géré, Bedon F, Pot D, Austerlitz F, Léger P, Kremer A, Plomion C.
DNA sequence polymorphism, linkage disequilibrium and haplotype structure in candidate genes of wood quality traits in maritime pine (Pinus pinaster Ait.).

R Minocha, Minocha SC, Long S.

Monitoring of environmental stress in forest trees using biochemical and physiological markers

Garaicoechea S et al.
Characterisation of local olive germplasm in the Basque Country in nothem Spain.

Annexe

GENOMIC RESEARCHES : NEW TOOLS FOR GENETIC IMPROVEMENT OF EUCALYPTS IN CONGO.

Vigneron Ph, Gion J-M & Verhaegen D
CIRAD Forêt. Montpellier (FRANCE)
UR2PI- Congo

Tree improvement is a long term and costly process which implies to address a lot of problems. The most difficult one is probably the precise assessment of the genetic value for a range of traits in a largely unpredictable environment. Growth, fibre yield and quality, wood physical and chemical properties, resistance to pests and diseases and cutting ability heavily rely to environmental conditions which disturb genetic prediction and gain. Development of molecular markers and functional genomic studies give new opportunities for a more direct and precise assessment of genotype values.

The Congolese plantations were established to supply pulp and paper industries. Due to severe environmental constraints, pure *Eucalyptus* species are not suitable for industrial plantations in Congo. Nevertheless, good yield can be achieved using hybrids such as *E. urophylla x E. grandis*. A breeding programme for eucalypts hybrids is conducted using Reciprocal Recurrent Selection as core of the breeding strategy (Vigneron, 1991). RRS is specially designed to improve hybrid varieties resulting from the combination of two unrelated populations (*e.g.* species) with complementary characteristics. Individuals from each pure parental population are crossed and performances of hybrid progenies are used for backward parent's selection as well as forward ortets selection. Several kinds of genotypes have to be assessed for a range of traits : families, parents through progeny and relatives, ortets and clones.

In order to improve selection accuracy as well as genetic diversity management through generations, molecular genetic studies were developed. This paper reports progress and perspectives for marker assisted selection in CIRAD eucalypts breeding programmes.

Genome organisation and genetic diversity.

Linkage maps of individual *E. urophylla* and *E. grandis* trees have been constructed using RAPD markers (Verhaegen and Plomion, 1996), microsatellites (from Embrapa) and candidate genes (Gion *et al.,* 2001) segregating in a pseudo-testcross configuration in a large F1 full sib family. The maps cover respectively 1193 cM and 1021 cM in 13 and 11 linkage groups for *E. urophylla* and *E. grandis* parents. In addition, 16 FS families were used to develop genetic maps for a larger sample of parents. Interspecific synteny was partially demonstrated between *E. urophylla* and *E. grandis*. It should facilitate genetic studies at subgenera level.

Widespread *Eucalyptus* species exhibit a high level of genetic diversity. Optimal constitution of collection and breeding populations with appropriate weight of relevant provenances needs a better knowledge of level and structure of genetic variability. DNA markers with neutral or unknown adaptative significance are used to identify pattern of variation resulting from time of isolation, mating system, gene flow, genetic drift and population structure. A monitoring of microsatellites and RAPD markers is under progress for both *E. urophylla* and *E. grandis* natural populations. These markers are able to give a picture of the evolution of genetic diversity across breeding cycles. As an example, gene flew within breeding seed orchard of *E. grandis* were assessed in Madagascar (Chaix, PhD thesis, *in prep.*). The study reveals a random-like mating system, as expected by breeders, but an unexpectedly high level of pollen pollution.

Beside neutral diversity, breeders are interested in allelic and functional diversity of useful genes controlling traits of interest. Functional genomics researches presented herein after will help to choose these genes.

Functional genomics

A global candidate gene investigation for eucalyptus is developed by CIRAD as a part of a larger European initiative. This approach involves three major steps : (i) identification of expressional candidate gene (CG), (ii) study of colocalisation between CG and quantitative trait loci (QTL) for particular pedigrees (two parents of an inter specific cross), and finally (iii) analysis of CG effect in breeding populations.

The identification of expressional CG can be approached at mRNA or protein levels. We plan to develop a proteomic approach in order to identify differentially expressed genes for traits of interest. Proteomics presents high potential for studying genes and genomes. It takes into account post-transcriptional events such as alternative splicing, translation regulation and allows the analyse of post-translational modifications. The study of proteome will be realized using the two dimensional electrophoresis for proteins separation and quantification combined with mass spectrometry methods for protein characterization. These approach will be developed for wood formation and vegetative propagation related traits.

The analyse of genetic architecture of quantitative trait *i.e.* number, localization and effect of QTL, is developed since 10 years. Using a large full sib family, a large number of QTL were found for a range of traits (Verhaegen *et al.*, 1997 ; Gion, 2001). Unfortunately, change in linkage disequilibrium between groups of genotypes, evolution of genetic control with age or season, genotype by environment interaction affect QTL stability and usefulness. The precise identification and isolation of the genes themselves becomes of major importance. CIRAD Forêt start a functional candidate genes approach with the well known genes involved in lignification process. The observed colocalisations between few genes and several QTL for wood quality such as lignin content and Syringyl/Guaïacyl ratio, are consistent with results from transgenesis studies. Development of electronic databases (from *Eucalyptus* or others angiosperms such as *Arabidopsis*), differential display studies of Expressed Sequence Tags or proteins will provide thousands of potential candidate genes. According to the existing knowledge of their roles in biochemical or regulatory pathways, some genes will be localized on genetic maps, searching for QTL colocalisations. Validation of CG heavily relies to adequate phenotype assessment of a number of genotypes in various environments for a range of traits such as wood physical or chemical properties. Adequate pedigrees, field trials and non destructive routine tests (NIR) are developed to support genomics studies.

The use of CG in MAS relies to the study of allelic variation in breeding population. Studies of single nucleotide polymorphisms (SNPs) of exonic regions of genes within breeding or natural populations enhance the possibility to find the most useful alleles for breeding. A first survey of SNPs is planned for few genes within the breeding population of *E. urophylla* and *E. grandis*. In a second step, the individuals displaying the different classes of alleles will be assessed for quality traits. SNPs as well as others markers will be used for MAS to i) set-up the core collection established on the basis of useful genetic variation, ii) choose parents for hybrid families, iii) select the ortets for next clonal tests.

Conclusion

Recent advances in molecular genetics allow a better knowledge of genes involved in complex quantitatifs characters : sequences, functions, expression level, localization... Going from lab to field and effective genetic and economic gain needs strong interconnection with traditional breeding and field trials. The current hybrid breeding strategy in Congo provides a lot of possibilities to do so : comprehensive collection of *E. urophylla*, *E. grandis*, *E. pellita* and others *Symphyomyrtus* species provenances, full-sib progeny tests for nearly two hundreds of parents in factorial mating designs

(more than 800 families), multisite clonal tests (hundreds of clones), family and clone by spacing and fertilisation interaction trials, which allow an integrative approach of *Eucalyptus* genome functions and diversity.

Gion JM (2000). Genetic architecture of quantitative traits in eucalyptus: from anonymous markers to candidate genes. PhD Thesis of the Université Rennes I.

Gion, Rech, Grima-Pettenati, Verhaegen, Plomion (2001). *Molecular Breeding*. 6 : 441-449

Gion J-M, Boudet C., Grima-Pettenati J, Ham Pichavant F, Plomion C, Baillères H and Verhaegen D (2001). Proceedings of the IUFRO Conference Developing The Eucalypt of the future. Valdivia Chili 10 to 15 september 2001.

Verhaegen D and Plomion C (1996). *Genome* 39, 1051-1061.

Verhaegen D, Plomion, Gion J-M, Poitel M, Costa P, Kremer A (1997 a). *Theoretical and Applied Genetics* 95, 597-608.

Vigneron Ph (1991). In 'Proceedings of IUFRO congress: Intensive Forestry, the role of eucalypts' pp. 345-360. (Durba

Annexe

Wood proteomics in maritime pine.

J.-M. Gion[1], C. Lalanne[2], H. Dumazet[2], C. Plomion[2]

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Differences in wood characteristics within a single tree are a common feature. These include: (i) variation within annual ring in temperate zones, i.e. early *vs* late wood, (ii) variation due to juvenile wood with extremely variable properties ranging from the core to the bark particularly in the early years of cambium activity, and (iii) variation between normal and reaction wood and more precisely between opposite wood *vs* compression wood. These six types of wood possess distinct chemical, anatomical and physical characteristics. This variability makes it possible to identify candidate genes involved in the genetic control of wood quality and end-product properties. Recent improvements in realisation of two-dimensional gel electrophoresis (e.g. immobilized pH gradient) and protein characterisation (mass spectrometry) allow considerable progress in proteome analysis. We have studied the proteome of differentiating xylem for the previous six types of wood. Proteins have been separated according to their isoelectric point (Isolelectric Focusing) using a 4 to 7 pH gradient and according to their molecular weights (SDS-PAGE) on a constant polyacrylamide gel. Along the six xylem conditions, a means of 600 proteins, for each condition comparisons, have been detected after colloidal blue staining. Around 200 spots have been cut of 2D gels for identification with mass spectrometry (MS/MS) analyses. Our first objective is to obtain a peptide map for differentiating xylem in maritime pine (i.e. reference gel with all spots characterized) and integrate all this information in a public database "PROTIC DB" under development. The second goal of our approach is to determine putative candidate genes/proteins which differentiate the studied xylem conditions. After spot quantification and volume normalization, the analysis of expression levels for the detected spots reveal that more significant differentially expressed proteins ($\alpha = 1\%$) have been detected between the juvenile *vs* late wood (30 % of the detected spots) in comparison to early wood *vs* late wood (20%) and opposite wood *vs* juvenile wood (11%).

Wood proteomics in maritime pine

J.-M. GION[1,2], C. LALANNE[2], H. DUMAZET[2], C. PLOMION[2]

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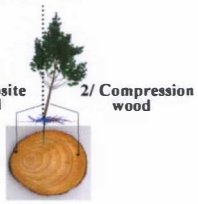
Introduction

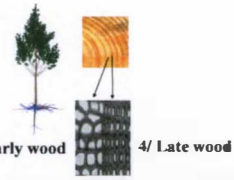
Differences in chemical, anatomical and physical characteristics of wood within a single tree could be explained by : (i) variations between normal and reaction wood, (ii) variations within annual ring, i.e. early *versus* late wood, and (iii) variations due to juvenile wood with extremely variable properties ranging from the core to the bark particularly in the early years of cambium activity. This variability makes it possible to correlate gene/protein expression profiles with wood properties, and ultimately to identify candidate genes involved in the genetic control of wood quality and end-product properties. Recent improvements in realisation of two-dimensional gel electrophoresis (e.g. immobilized pH gradient) and protein characterisation (mass spectrometry) allow considerable progress in proteome analysis. These advances, although not very used in plant genomics, afford opportunities to have an overlook of gene product expression and address physiological question at a level that is much relevant than mRNA.

Material and methods

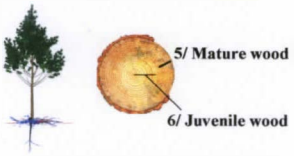
Six types of wood :



• On 14 years-old clones, sampling differentiating xylem were collected on two years bended trees (opposite and compression sides).



• On 14 years-old clones, samples of differentiating xylem were collected on April, 1st and September, 1st.



• On 35 years-old clones, sampling differentiating xylem at the top (juveline wood) and the bottom (mature wood) of the bole.

Two dimensional gel electrophoresis :

Protein extraction → **1st dimension : Isoelectric Focusing**
- Using a 4 to 7 pH gradient
- Using 24 cm IPG strip

→ **2nd dimension : SDS-PAGE** (15KD < Molecular Weight < 90KD)

Constant polyacrylamide gel have been used

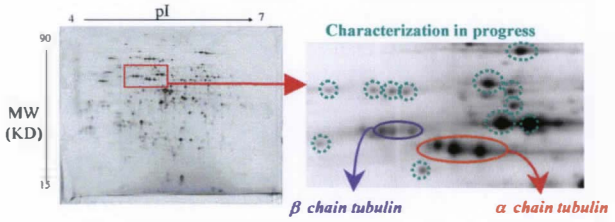
Image Analyses :

Spot quantification analysis were carried out with Image Master 2D software (Amersham) after a colloïdal blue staining.

Results

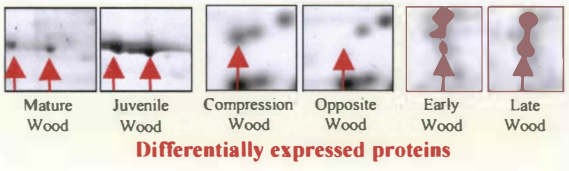
1/ A xylem peptide map for maritime pine :

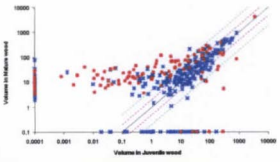
- Characterization in progress for 300 xylem proteins using two mass spectrometry methods (MALDI-TOF and MS/MS).



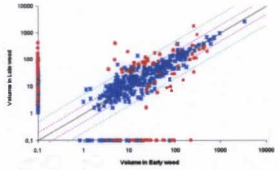
2/ Identification of differentially expressed proteins :

- Significant differentially expressed spots are represented in red ($p<0.01$).
- Non Significant differentially expressed spots are represented in blue.

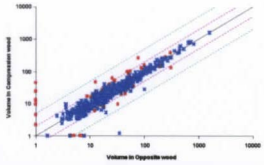




30% of analysed proteins were differentially expressed.



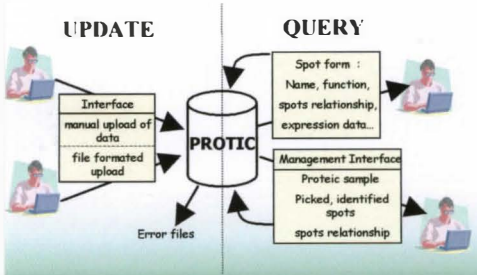
20% of analysed proteins were differentially expressed.



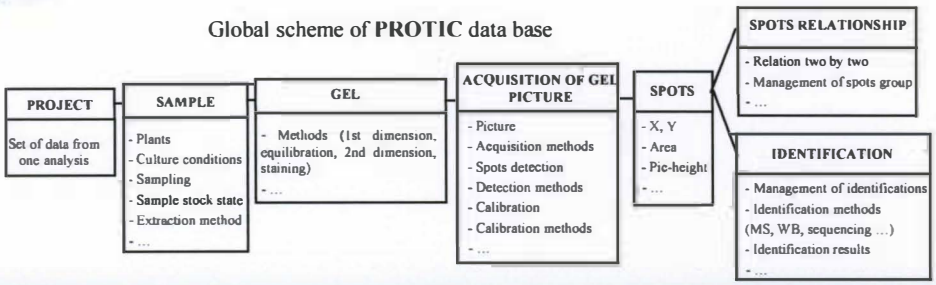
11% of analysed proteins were differentially expressed.

3/ Integrate the results in a public database :

Modelisation of **PROTIC** data base



Global scheme of **PROTIC** data base



Acknowledgements: This work is supported by European Union (QLRT-1999-00942, GEMINI) and Région Aquitaine (CCRRDT 20000307007).

Genomic researches : New tools for genetic improvement of eucalypts.

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Jean-Marc Gion
Daniel Verhaegen
Gilles Chaix

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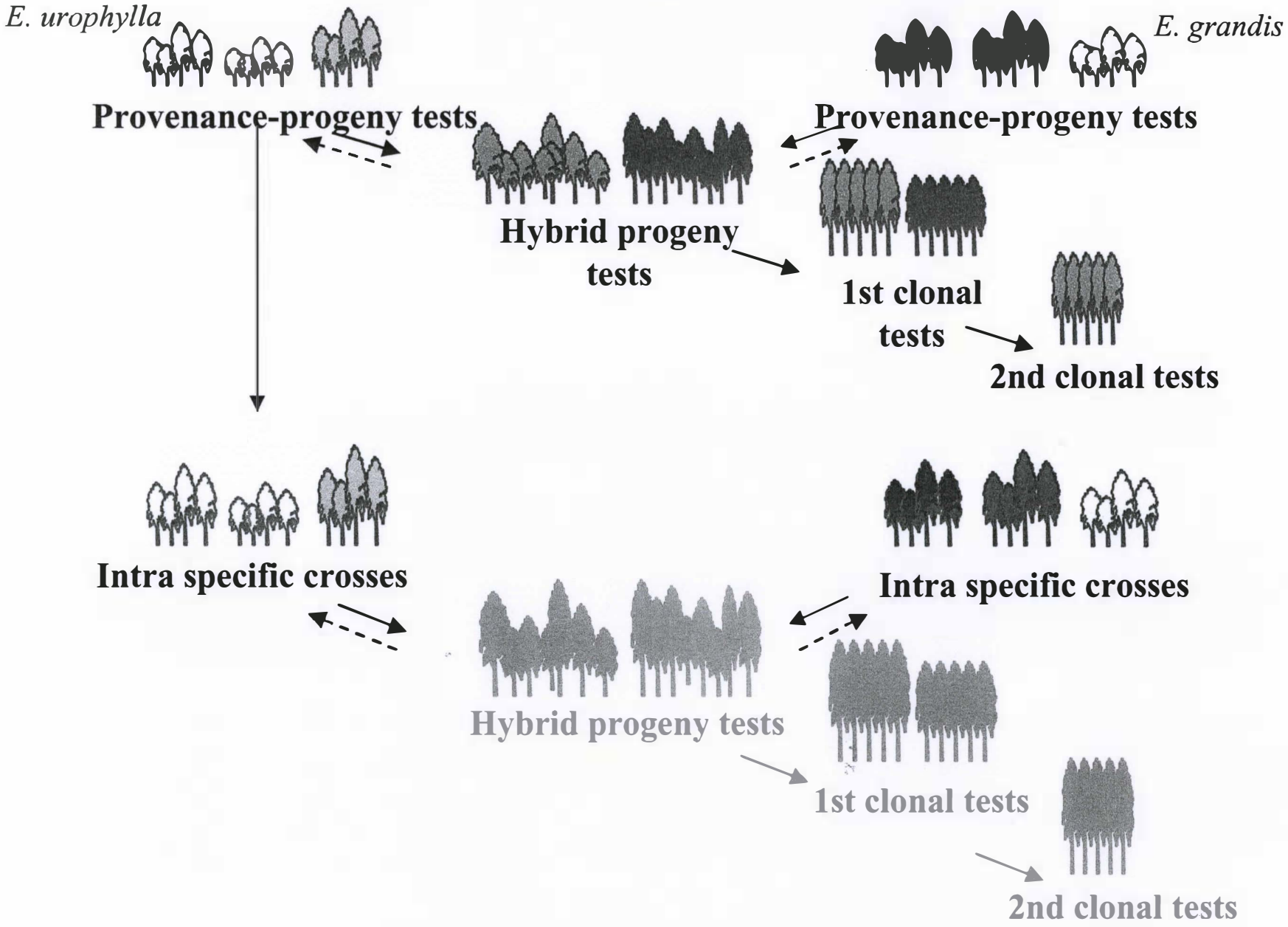
CIRAD

Biofor02
Vitoria Gasteiz

Recurrent Reciprocal Selection

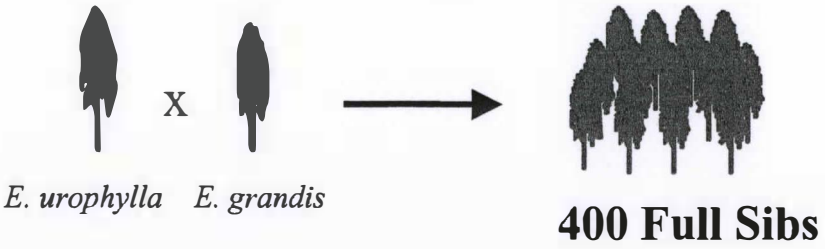


- Genome organization and genetic diversity

- Gene expression and genetic architecture of quantitative traits

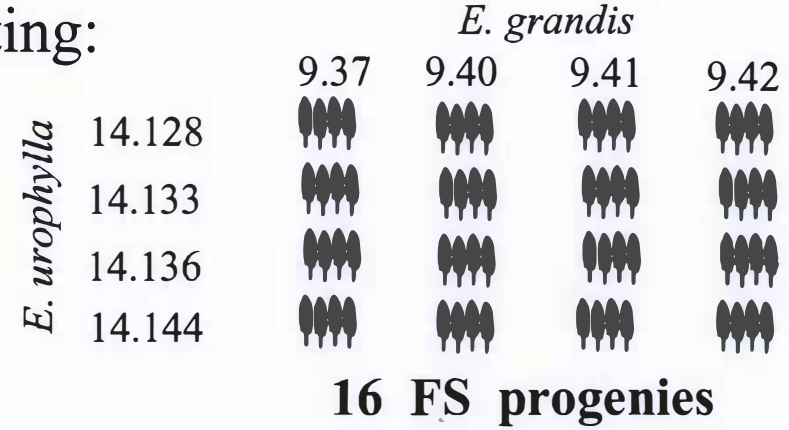
Genetic mapping

- Full sib familly:

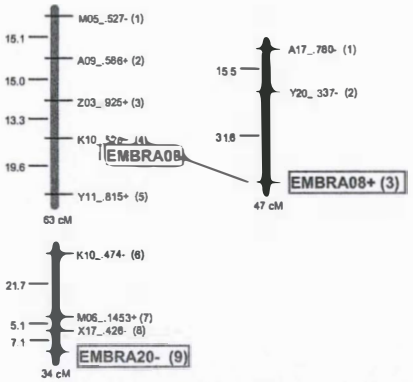


⇨ Two saturated genetic maps

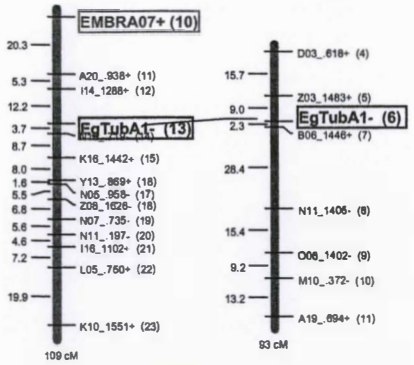
- 4x4 Factorial mating:



⇨ Eigth partial genetic maps



GROUP 1



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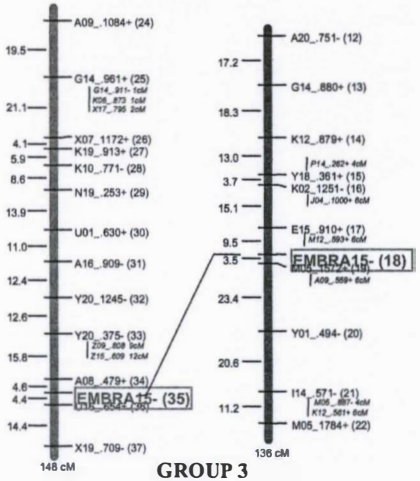

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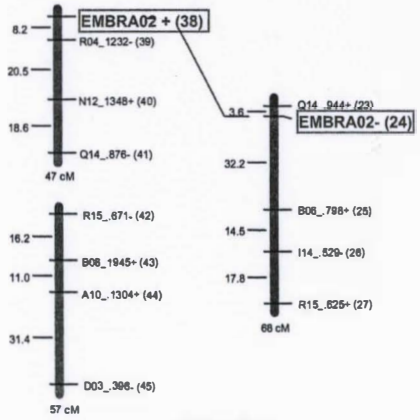

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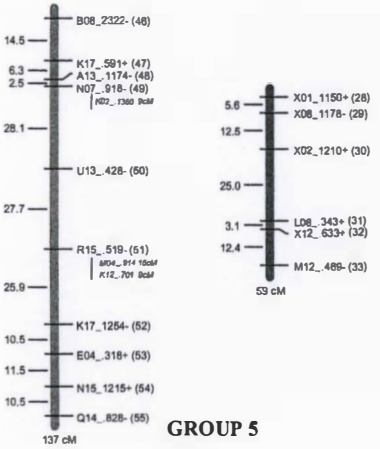
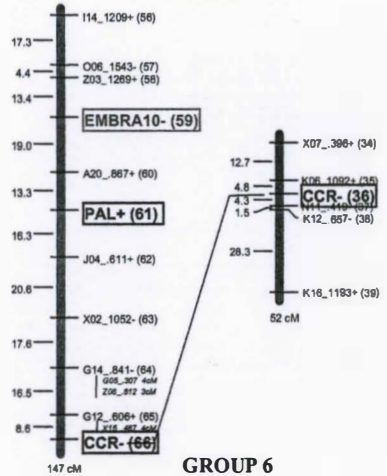


GROUP 5



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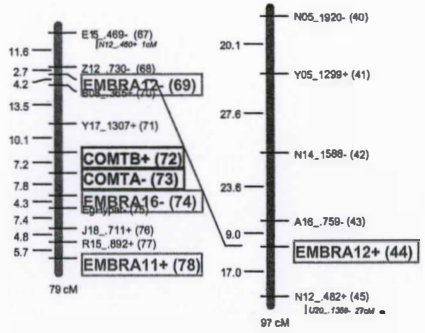
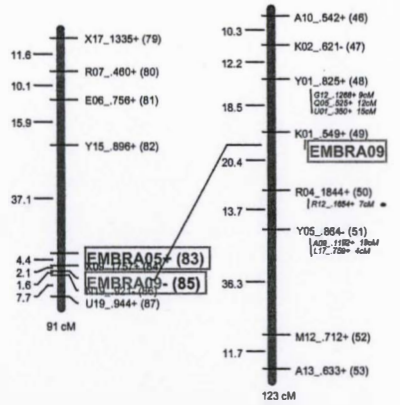
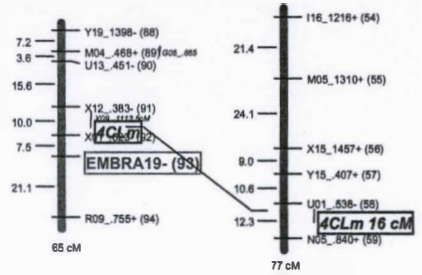


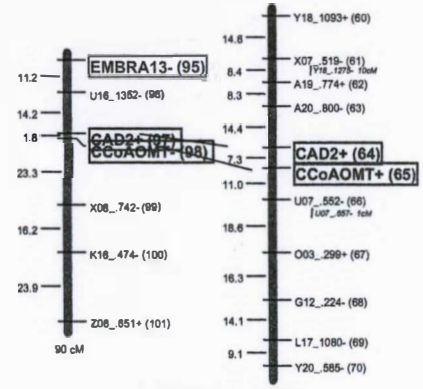
GROUP 7



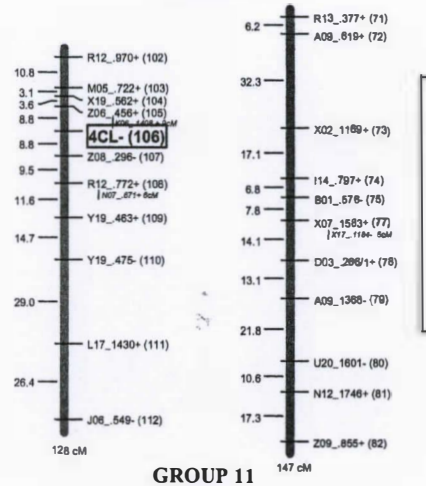
GROUP 8



GROUP 9



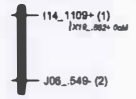
GROUP 10



GROUP 11

LEGEND

E. urophylla ***E. grandis***



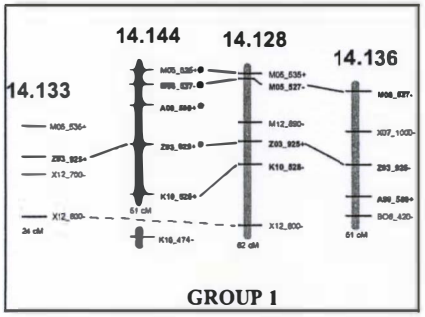
EMBRA13- (95) SSR

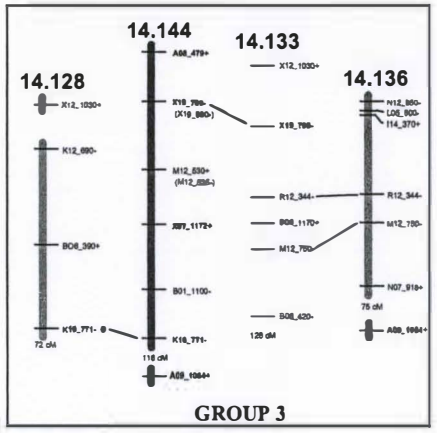
4CL- (106) GENES

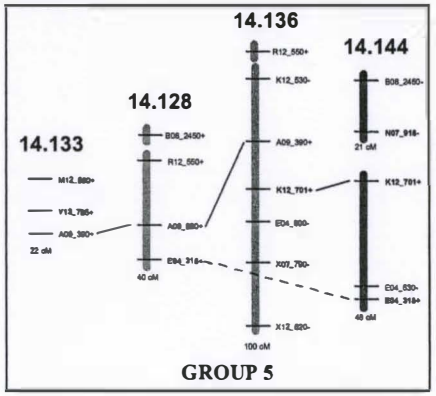
R13_.377+ (71) RAPD

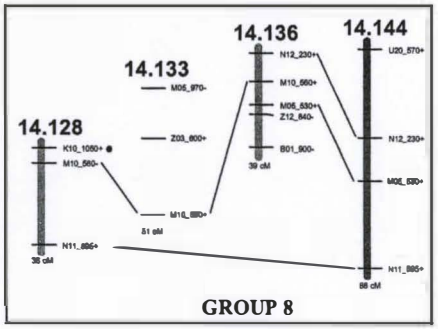
Partial genetic maps for 4 *E. urophylla* genotypes

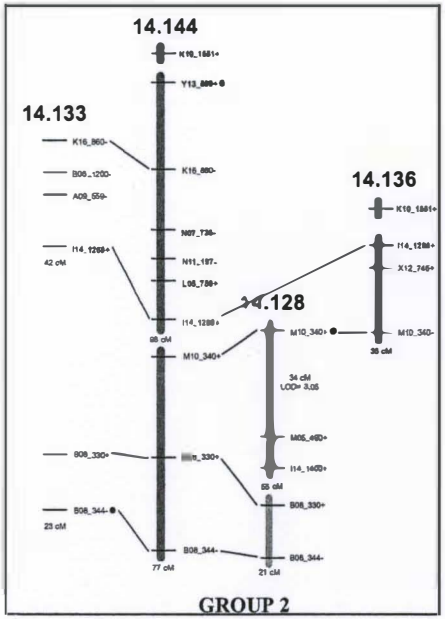
Percentage of coverage ≈ 50%

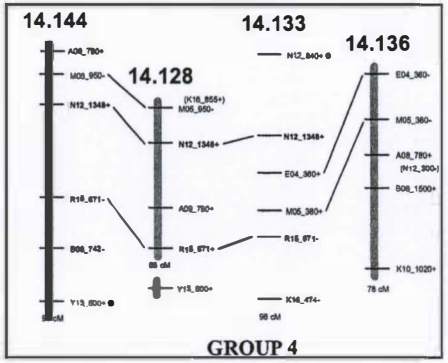


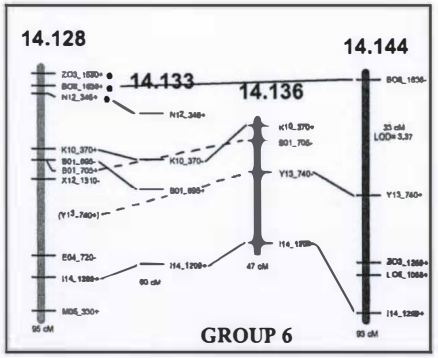


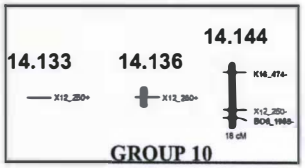


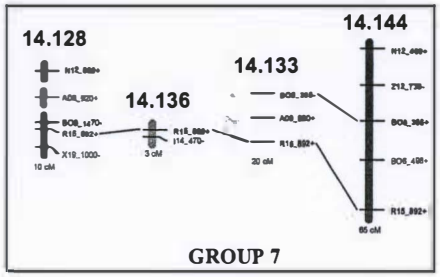


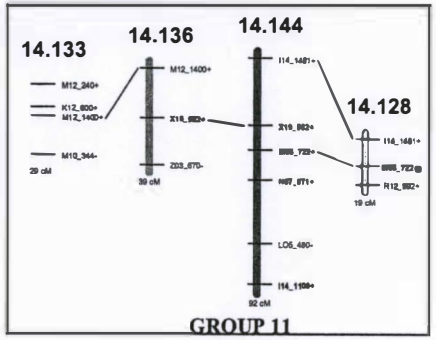






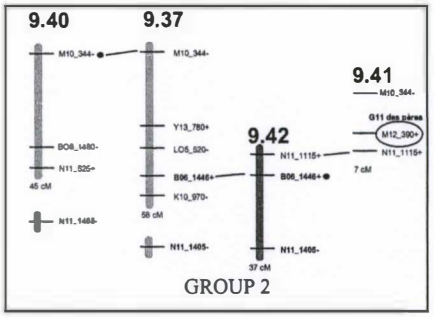




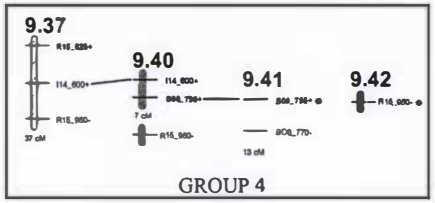


Partial genetic maps for 4 *E. grandis* genotypes

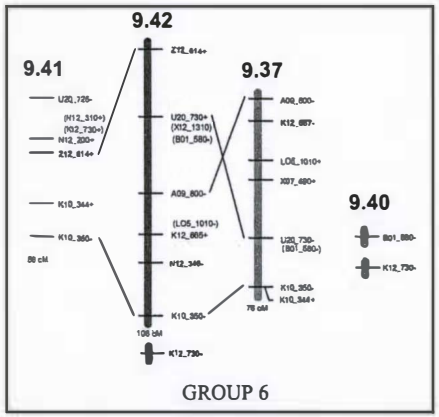
Percentage of coverage ≈ 50%



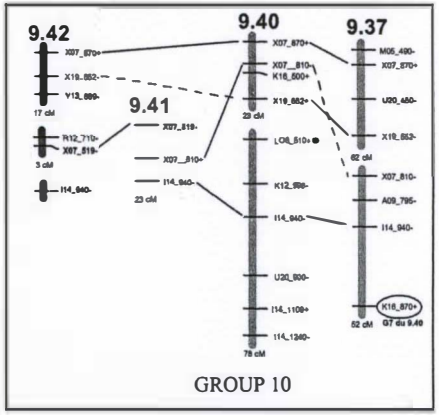
GROUP 2



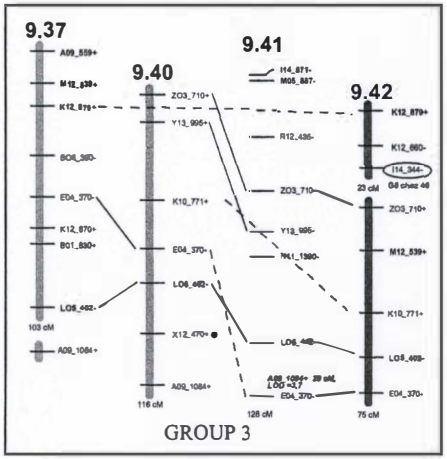
GROUP 4



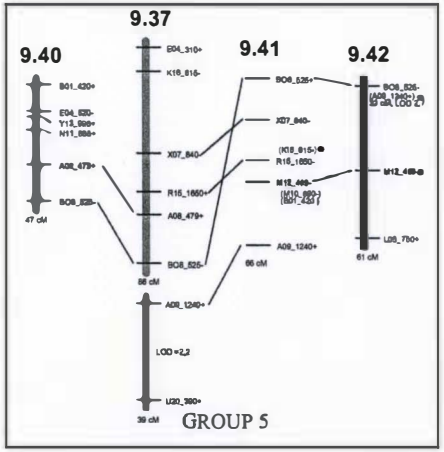
GROUP 6



GROUP 10



GROUP 3



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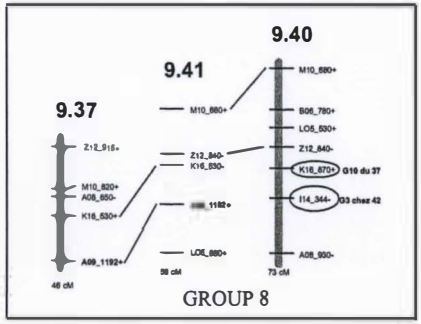

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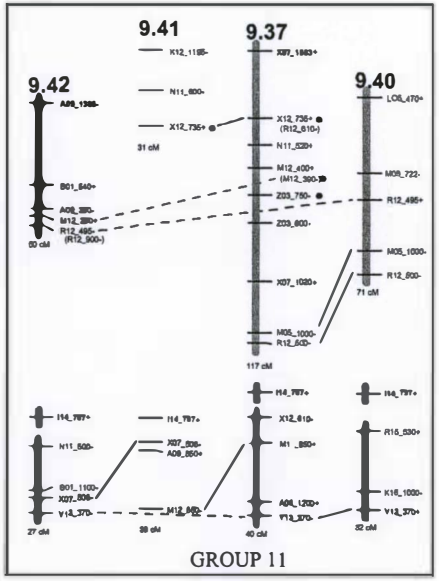
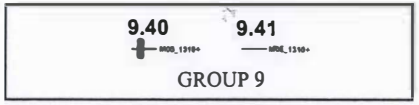


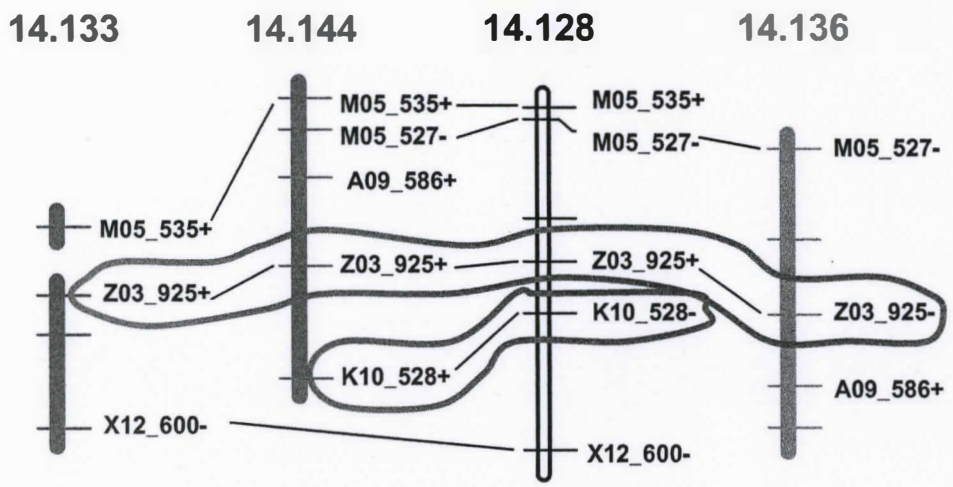
GROUP 11



GROUP 9

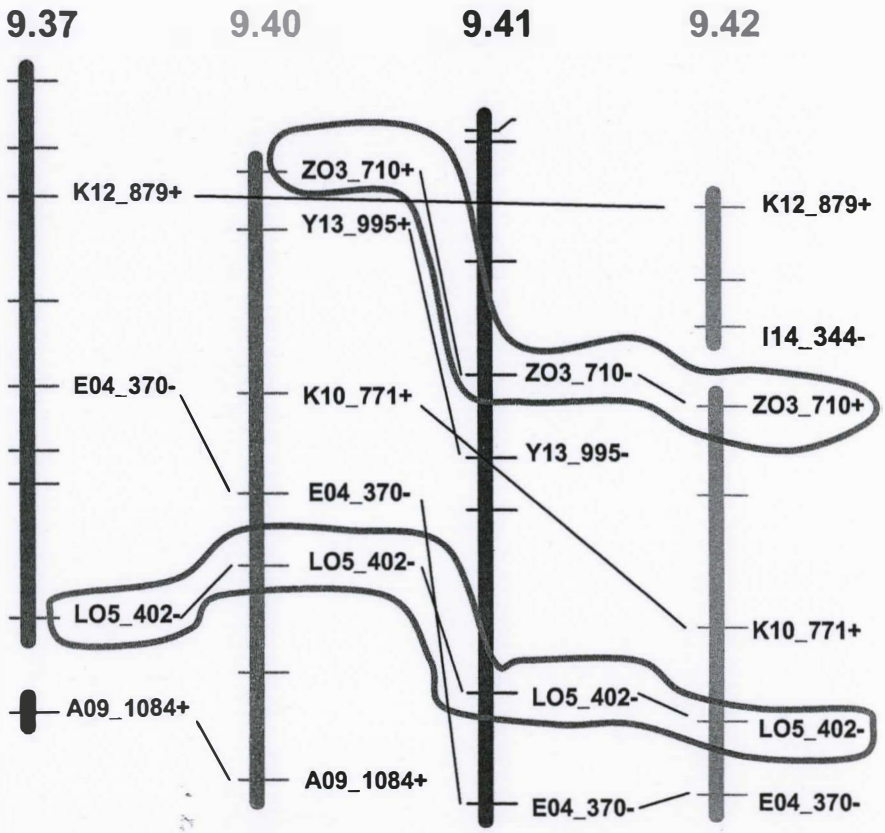
Homologous linkage groups

4 *E. urophylla* genotypes



Linkage group 1

4 *E. grandis genotypes*



Linkage group 3

Homologies with others *Eucalyptus urophylla* and *E. grandis* maps

Homologies with several markers

Homologies with only one marker

No homology

SSR Markers	Linkage group number			
	CIRAD		**Brondani *et al.*(1998)**	
	E. urophylla	*/ E. grandis*	*E. urophylla*	*/ E. grandis*
EMBRA12	GL7	GL7	GL1	GL1
EMBRA16	GL7	-	GL1	GL1
EMBRA11	GL7	-	GL1	GL1
EMBRA5	GL8	-	GL5	GL5
EMBRA9	GL8	GL8	GL5	GL5
EMBRA15	GL3	GL3	GL8	GL8
EMBRA2	GL4a	GL4	-	GL11
EMBRA10	GL6	-	GL10	GL10
EMBRA19	GL9	-	GL4	GL4
EMBRA7	GL2	-	GL9	-
EMBRA13	GL10	-	GL	GL9
EMBRA20	GL1b	-	-	GL7
EMBRA8	GL1a	GL1a	-	GL6

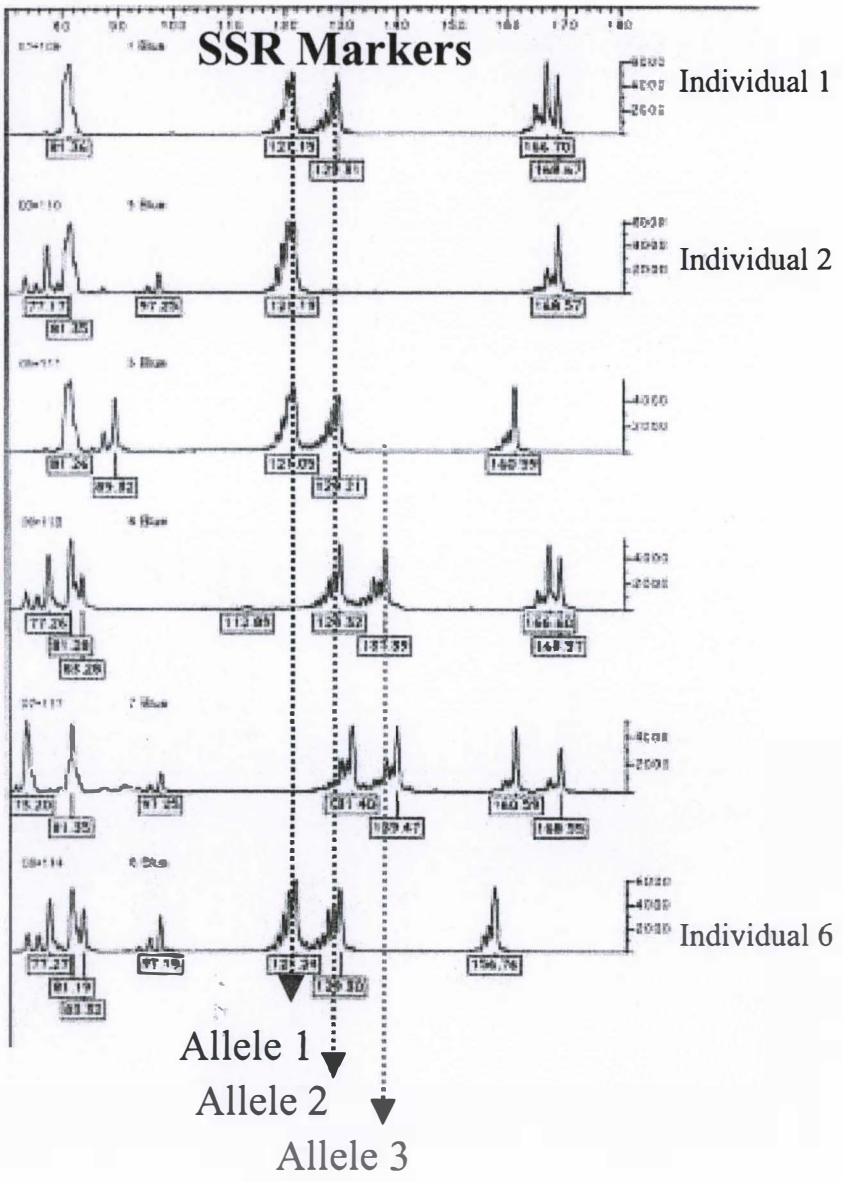
Towards a consensus genetic map for *Symphyomyrtus* subgenus

- Mapping of new codominant markers
  - Single Sequence Repeat markers
  - Expressed Sequence Tags

- Development of Bacterial Artificial Chromosome library for the future

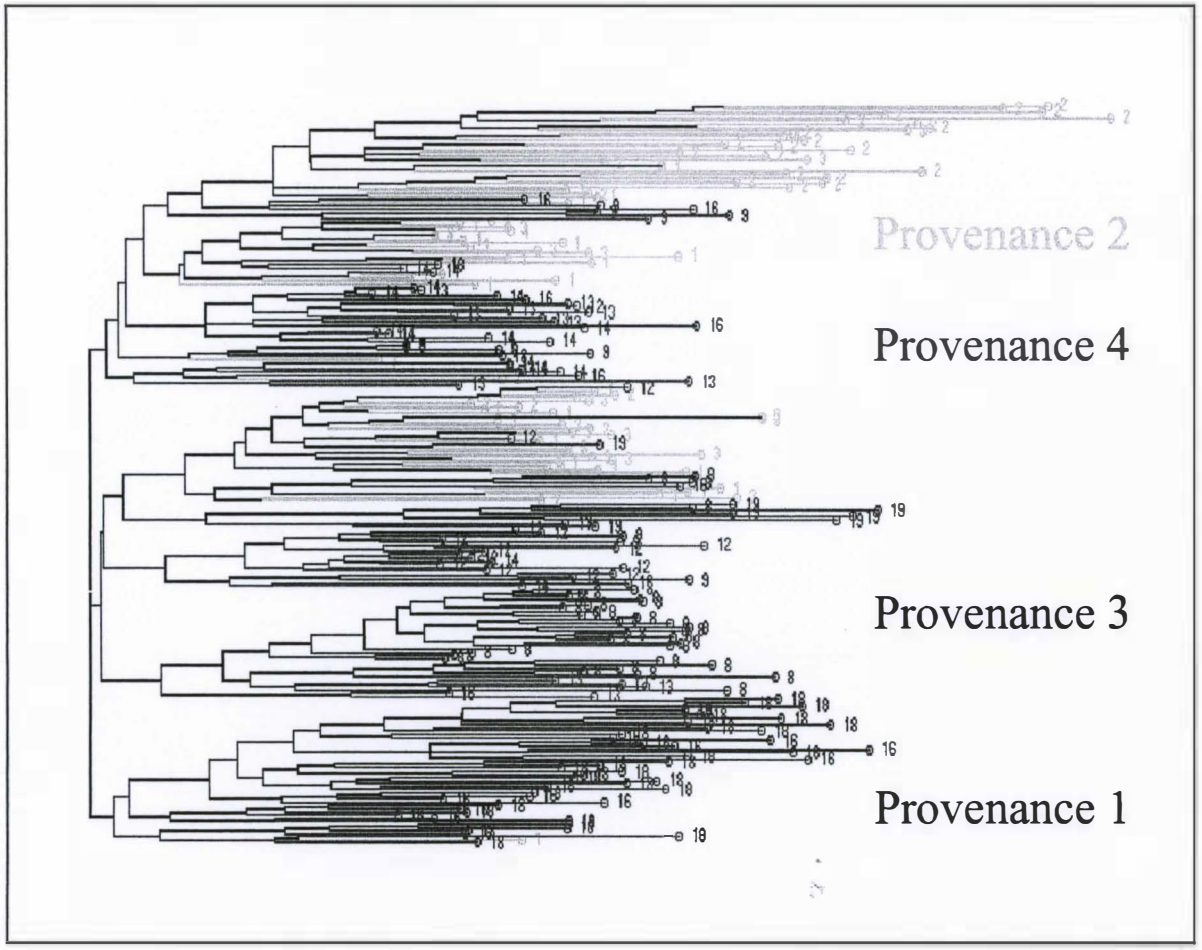
Genetic diversity and gene flow

Eucalyptus grandis
breeding population



Genetic Diversity Structure

E. grandis breeding population based on several provenances



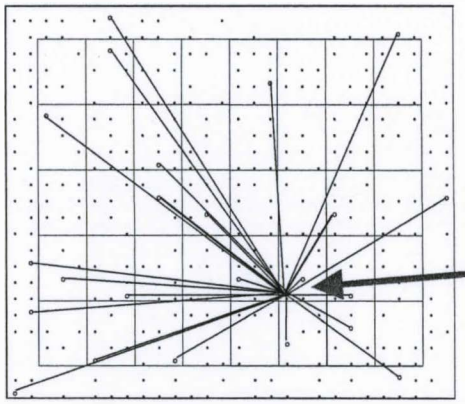
⇒ New organization of breeding groups

Gene Flow

- Balanced contribution of the different genotypes
- Low level of self pollination: 3 %
- Random like mating system

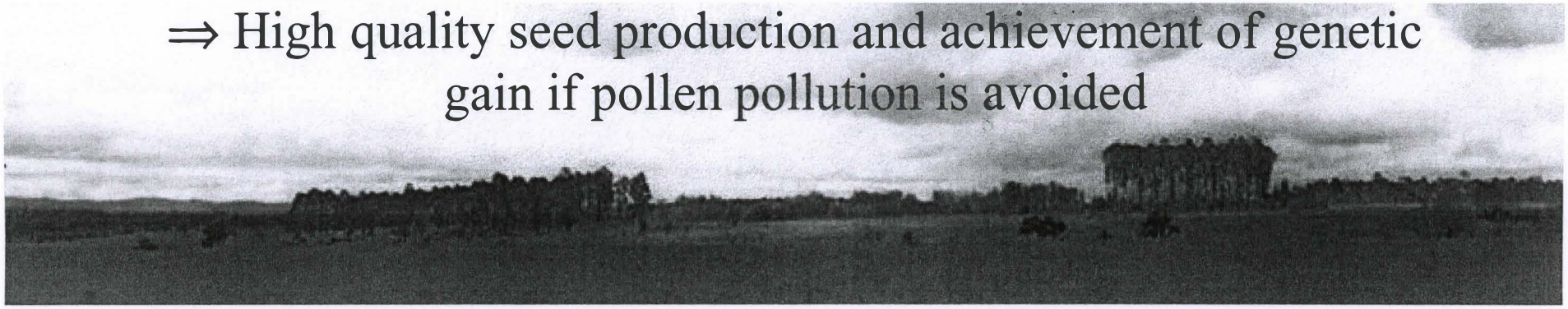
High level of undesirable alien pollen

48% ⟹



Mother tree 76

⟹ High quality seed production and achievement of genetic gain if pollen pollution is avoided



- Genome organization and genetic diversity

- Gene expression and genetic architecture of quantitative traits

Three steps in candidate gene approach

Expressional candidate gene (CG)

⇒ Transcriptomic studies (messenger RNA)

⇒ Proteomic studies (proteins)

Functional candidate gene

⇒ Genetic mapping

⇒ QTL detection

⇒ PQL detection

Validation of allelic effects

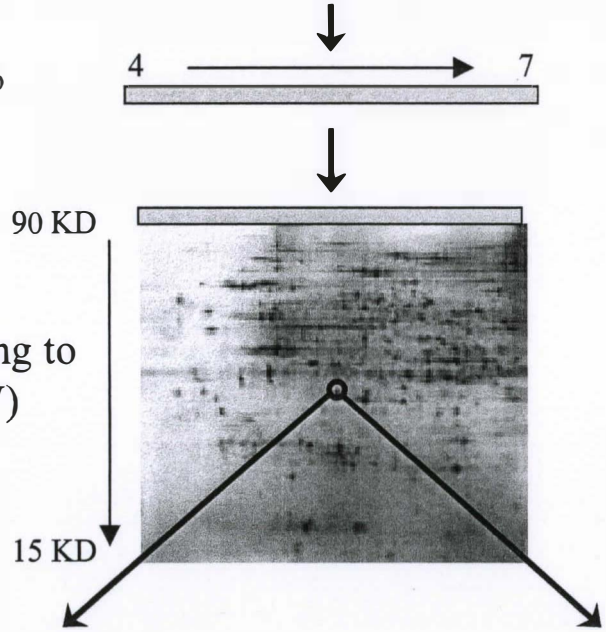
⇒ Test gene effect in breeding populations (SNPs…)

Proteomic approach

Bidimensional Electrophoresis :

Proteins extraction

Separating polypeptides according to their isoelectric point



First dimension :
Isoelectric focusing

Separating polypeptides according to their molecular weights (MW)
15KD < MW < 90KD

Second dimension :
SDS-PAGE

Protein Identification
(Mass spectrometry)

Protein Quantification
(Differential expression analysis)

A « pool » of candidate proteins/genes

Why functional CG are necessary ?

QTL Unstability with

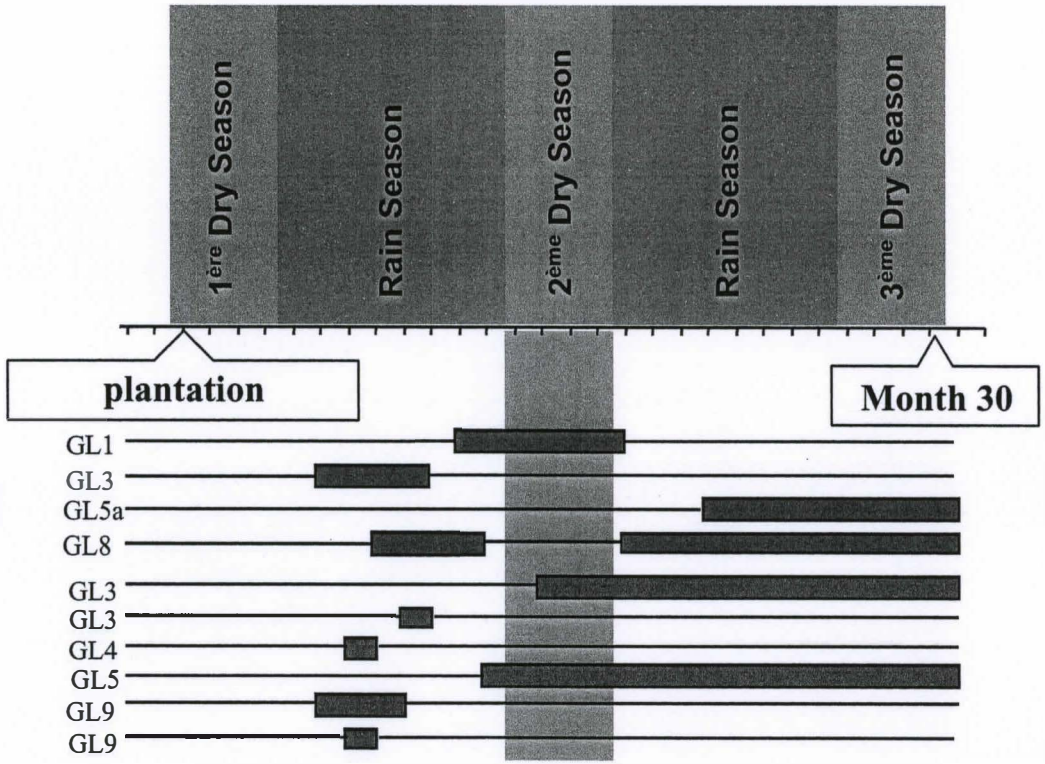
Age

Environment

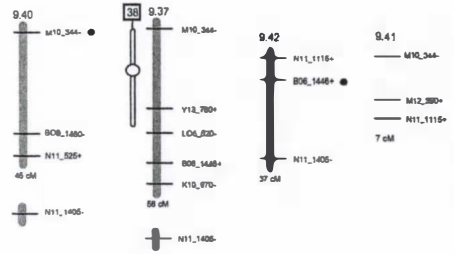
Pedigrees / populations

QTL change with age

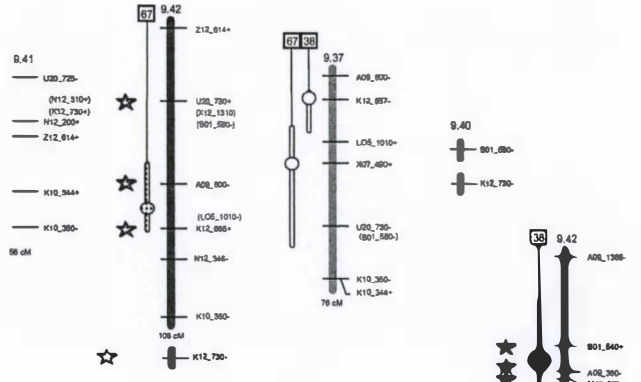
Seasons:



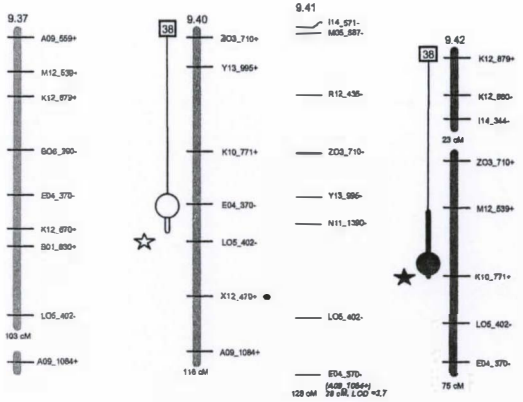
QTL detection for different genotypes



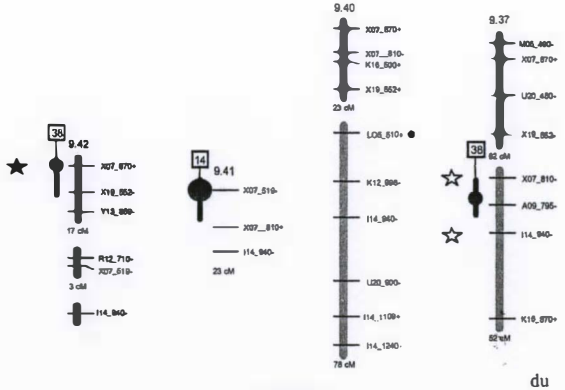
GROUP 2



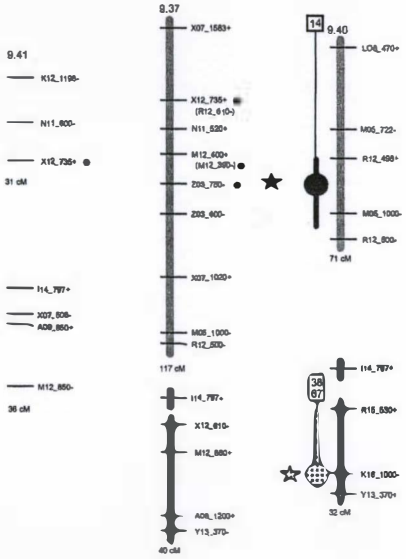
GROUP 6



GROUP 3



GROUP 10



GROUP 11

Functional CG

Genetic Mapping

- Lignification genes
- Expressed sequence tag (expressional CG)…

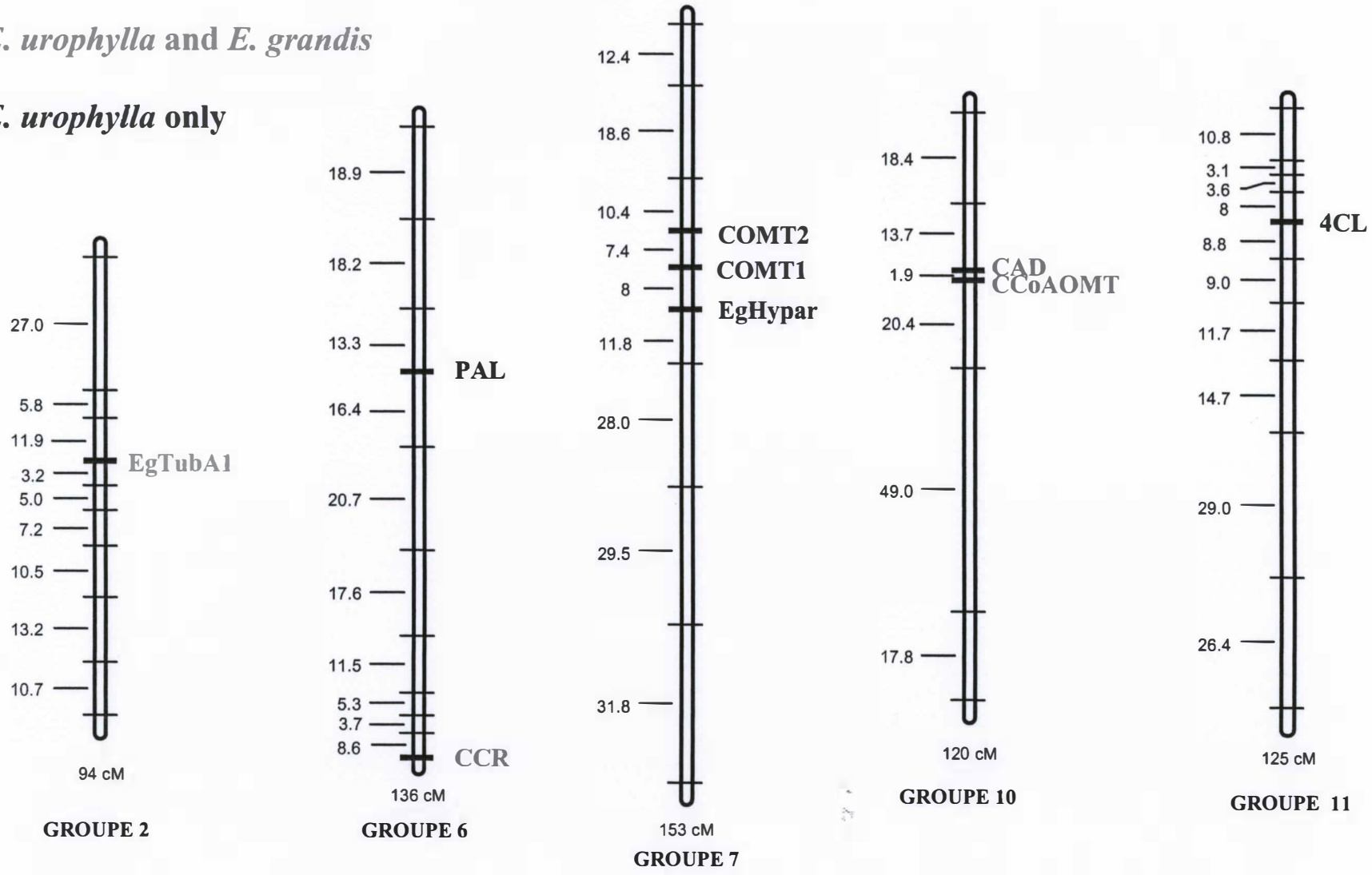
Quantitative trait Loci analysis

- Several wood properties
- Other quantitative traits

Genetic mapping on existing maps:

For *E. urophylla* and *E. grandis*

For *E. urophylla* only



E. urophylla

QTL Detection

- Strong effect of QTL

  4 QTL explaining 41 % of lignin content variation

  3 QTL explaining 33 % of S/G ratio variation

- Association between traits

  Growth and wood quality

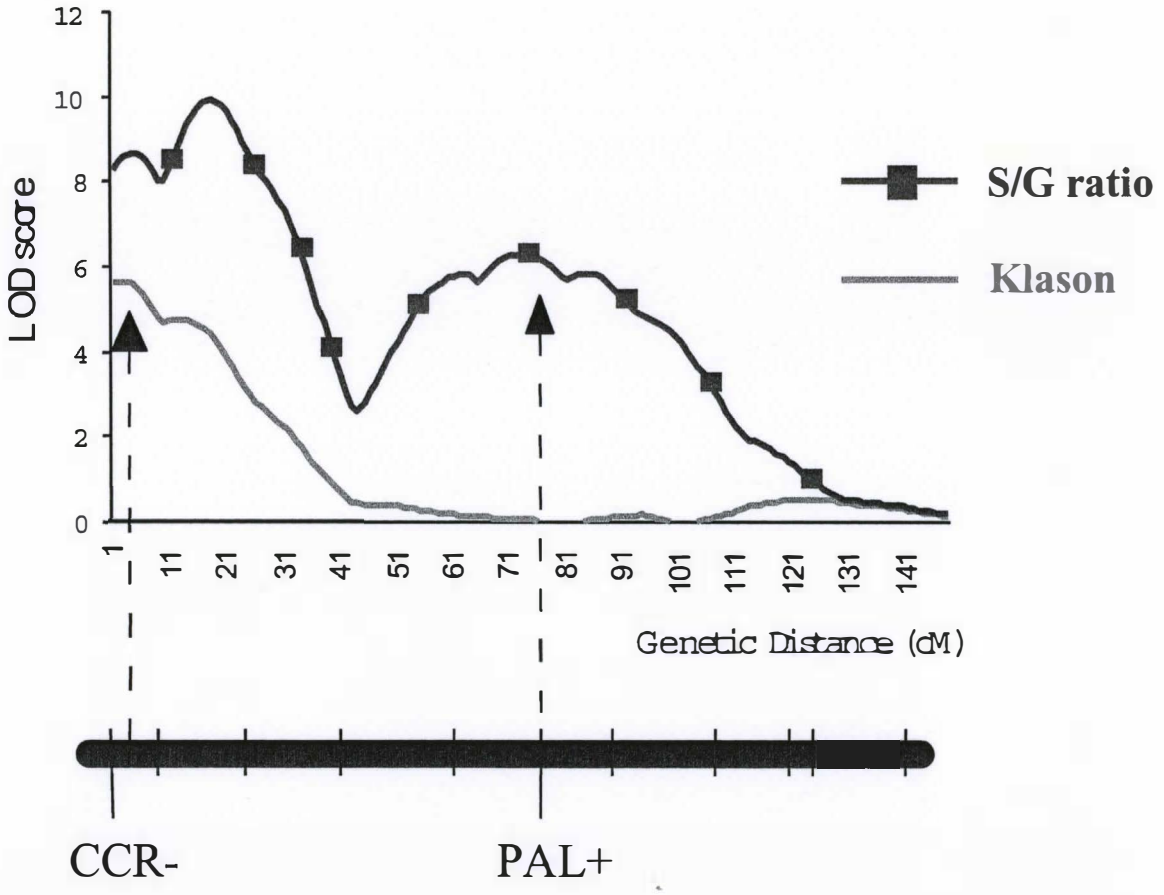
- Colocalisation genes - QTL

Joint segregation Genes / Quantitative Trait:

CCR and PAL

Linkage group 6

E. urophylla



⇒ Strong association QTL - gene

- Evidence for 4 candidate genes

  - CCR ⇒ lignin content, S/G ratio
  - PAL ⇒ S/G ratio
  - 4CL ⇒ wood density
  - COMT⇒ spliting index

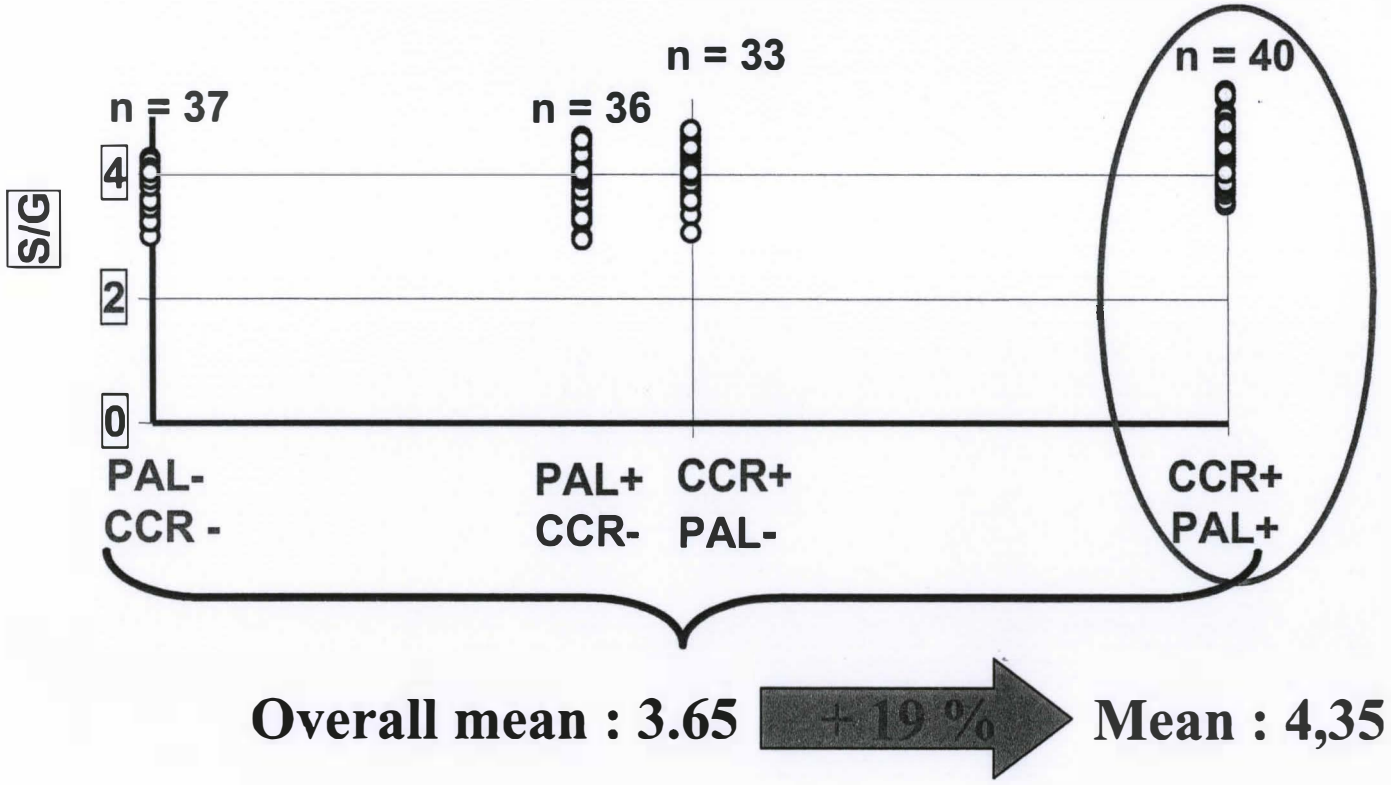
  ⇒ consistent with results from transgenesis

- No colocalisation with CAD and CCoAOMT

Allelic effect within a family

Combined effect of CCR and PAL favorable alleles

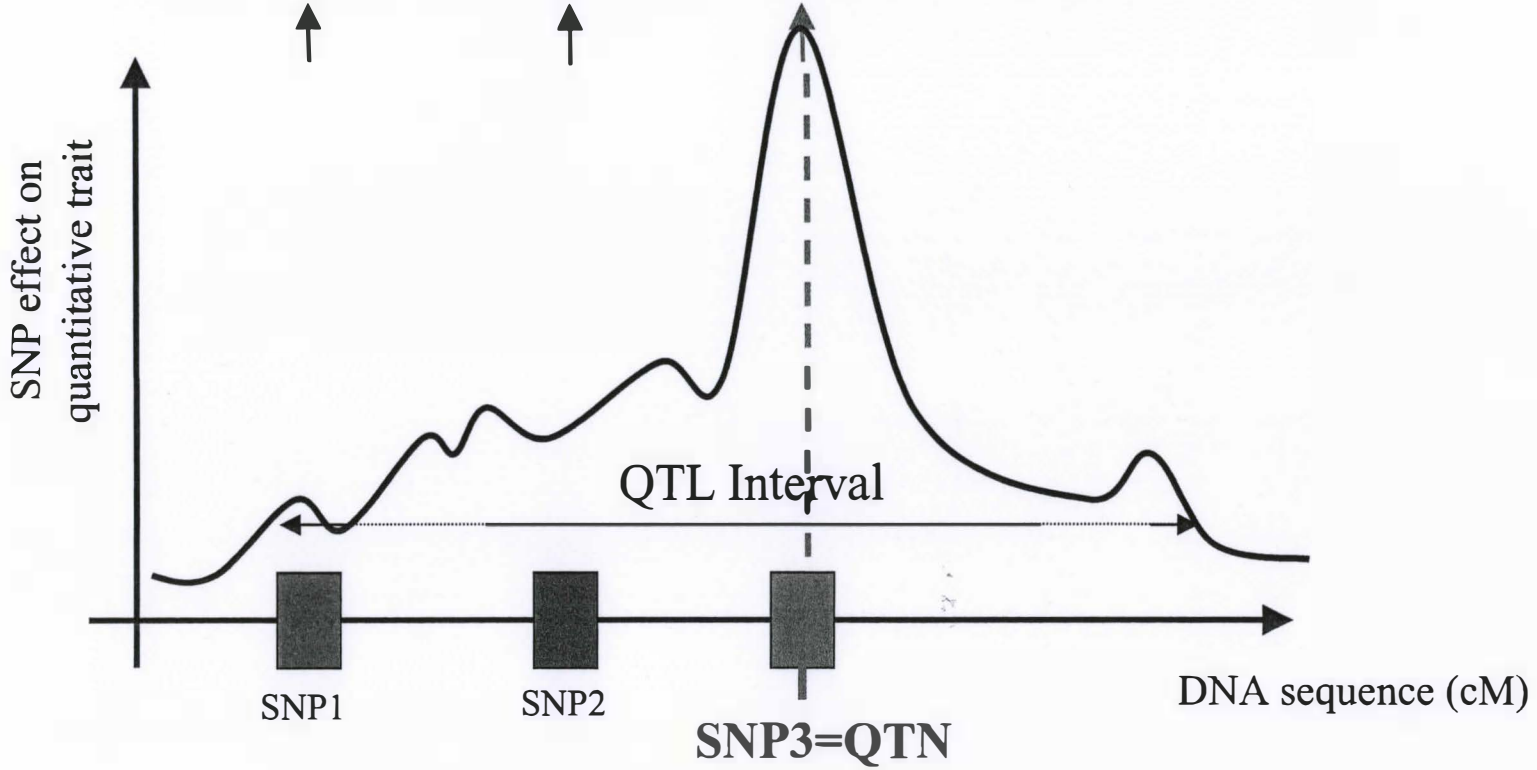
S / G ratio variation in a full sib family of *E. urophylla* x *E. grandis*



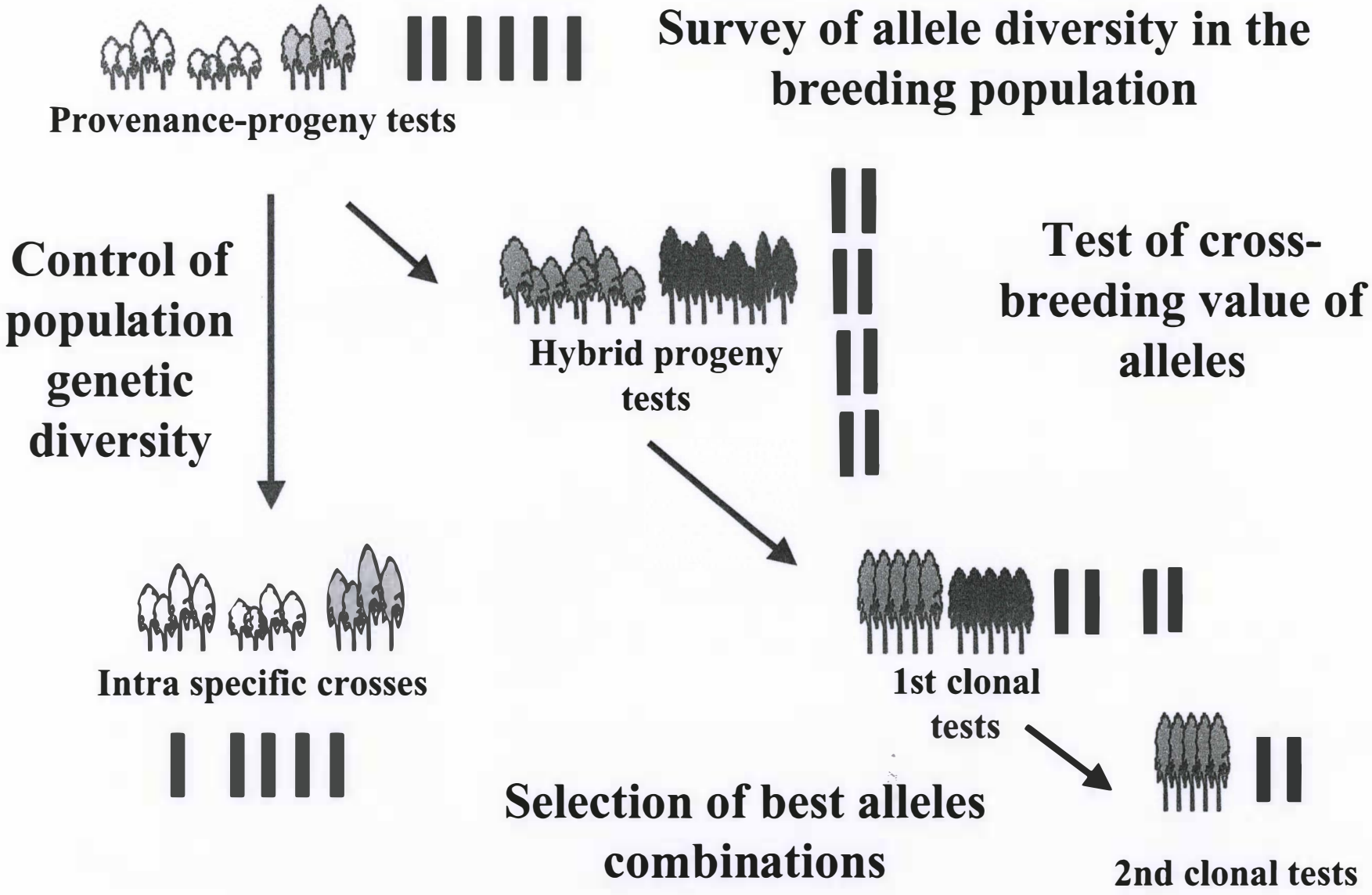
Gene variation effect within breeding population ?

Single Nucleotide Polymorphism

...GGCTG...GTGCAT...CTAGGCCTAG...
...GGCAG...GTGGAT...CTAGGCCTAG...
...GGCTG...GTGCAT...CTTGGCCTAG...
...GGCAG...GTGCAT...CTTGGCCTAG...
...GGCAG...GTGGAT...CTTGGCCTAG...



Towards a Marker Assisted Selection



A considerable amount of field trials

- **Provenance-progeny trials**

  55 000 individual *E. urophylla*, *E. grandis* and *E. pellita* trees

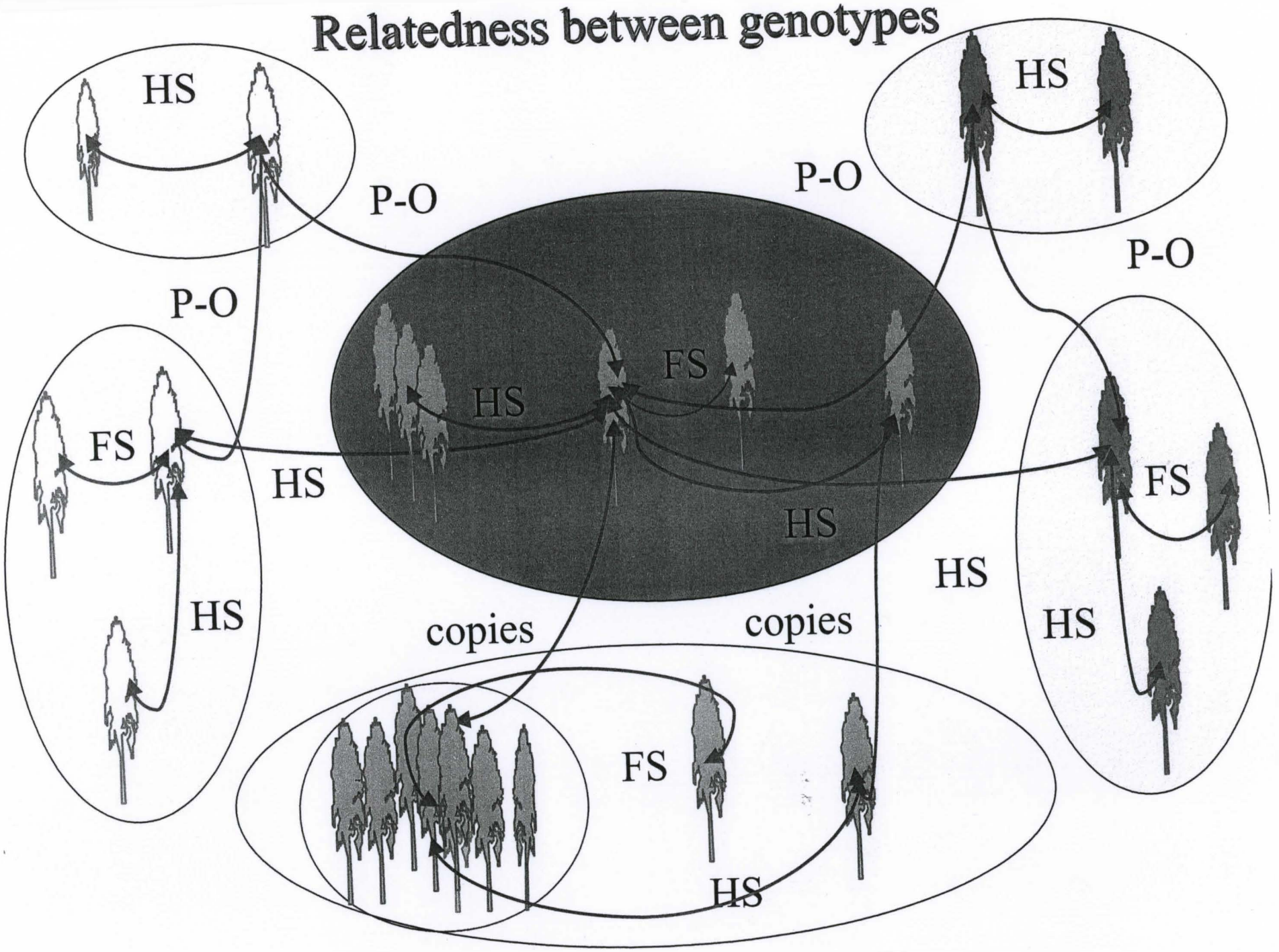
- **Hybrid progeny trials** (factorial mating designs)

  - 200 parent trees in tests

  - 800 full sib hybrid families (50 to 100 trees)

- **Clonal tests** (multisite)

  300 *E. urophylla* x *E. grandis* clones in long term tests
  (3 sites x 3 reps x 9 ramets)


Relatedness between genotypes
HS
P-O
P-O
HS
P-O
P-O
FS
HS
FS
HS
HS
HS
HS
FS
HS
copies
copies
FS
HS

Gene / allele effect through pedigrees